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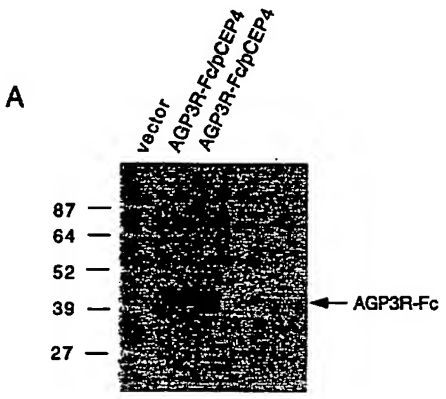
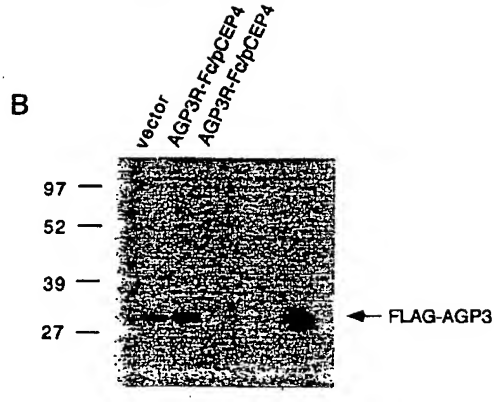
(54) Title: RECEPTOR FROM TNF FAMILY





(57) Abstract: A member of the tumor necrosis factor family and its receptor are described. This member is primarily expressed in B cells and its expression correlates to increases in the number of B cells and immunoglobulins produced. The natural, preferred human ortholog is here called AGP-3R. The protein is a type III transmembrane protein and has an amino terminal extracellular domain, a transmembrane domain, and a carboxy terminal intracellular domain. AGP-3R-related proteins of the invention may be membrane-associated or in soluble form, recombinantly produced or isolated after natural production. The invention provides for nucleic acids encoding such AGP-3R-related proteins, vectors and host cells expressing the polypeptides, and methods for producing recombinant proteins. Antibodies or fragments thereof that specifically bind the proteins are also provided.

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RECEPTOR FROM TNF FAMILY

This application claims the benefit of U.S. Provisional Application Serial No. 60/181,800, filed February 11, 2000, which is hereby incorporated by reference.

Field of the Invention

The present invention relates to proteins that are involved in inflammation and immunomodulation, particularly in B cell growth, survival, or activation. The invention further relates to proteins related to the tumor necrosis factor (TNF)/nerve growth factor (NGF) superfamily and related nucleic acids, expression vectors, host cells, and binding assays. The specification also describes compositions and methods for the treatment of immune-related and inflammatory, autoimmune and other immune-related diseases or disorders, such as rheumatoid arthritis (RA), Crohn's disease (CD), lupus, and graft versus host disease (GvHD).

Background of the Invention

After years of study in necrosis of tumors, tumor necrosis factors (TNFs) α and β were finally cloned in 1984. The ensuing years witnessed the emergence of a superfamily of TNF cytokines, including fas ligand (FasL), CD27 ligand (CD27L), CD30 ligand (CD30L), CD40 ligand (CD40L), TNF-related apoptosis-inducing ligand (TRAIL, also designated AGP-1), osteoprotegerin binding protein (OPG-BP or OPG ligand), 4-1BB ligand, LIGHT, APRIL, and TALL-1. Smith et al. (1994), Cell 76: 959-962; Lacey et al. (1998), Cell 93: 165-176; Chichepotiche et al. (1997), J. Biol. Chem. 272: 32401-32410; Mauri et al. (1998), Immunity 8: 21-30; Hahne et al. (1998), J.Exp. Med. 188: 1185-90; Shu et al. (1999), J. Leukocyte Biology 65: 680-3. This family is unified by its structure, particularly at the C-terminus. In addition, most members known to date are expressed in immune compartments, although some members are also expressed in

other tissues or organs, as well. Smith et al. (1994), Cell 76: 959-62. All ligand members, with the exception of LT-α, are type II transmembrane proteins, characterized by a conserved 150 amino acid region within C-terminal extracellular domain. Though restricted to only 20-25% identity, the conserved 150 amino acid domain folds into a characteristic β-pleated sheet sandwich and trimerizes. This conserved region can be proteolyticaly released, thus generating a soluble functional form. Banner et al. (1993), Cell 73: 431-445.

Many members within this ligand family are expressed in lymphoid enriched tissues and play important roles in the immune system development and modulation. Smith et al. (1994). For example, TNFα is mainly synthesized by macrophages and is an important mediator for inflammatory responses and immune defenses. Tracey & Cerami (1994), Annu. Rev. Med. 45: 491-503. Fas-L, predominantly expressed in activated T cell, modulates TCR-mediated apoptosis of thymocyts. Nagata, S. & Suda, T. (1995) Immunology Today 16: 39-43; Castrim et al. (1996), Immunity 5: 617-27. CD40L, also expressed by activated T cells, provides an essential signal for B cell survival, proliferation and immunoglobulin isotype switching. Noelle (1996), Immunity 4: 415-9.

The cognate receptors for most of the TNF ligand family members have been identified. These receptors share characteristic multiple cysteine-rich repeats within their extracellular domains, and do not possess catalytic motifs within cytoplasmic regions. Smith et al. (1994). The receptors signal through direct interactions with death domain proteins (e.g. TRADD, FADD, and RIP) or with the TRAF proteins (e.g. TRAF2, TRAF3, TRAF5, and TRAF6), triggering divergent and overlapping signaling pathways, e.g. apoptosis, NF-κB activation, or JNK activation. Wallach et al. (1999), Annual Review of Immunology 17: 331-67. These signaling events lead to cell death, proliferation, activation or differentiation. The expression profile of each receptor member varies. For example, TNFR1 is

expressed on a broad spectrum of tissues and cells (13); whereas the cell surface receptor of OPGL is mainly restricted to the osteoclasts. Hsu et al. (1999) Proc. Natl. Acad. Sci. USA 96: 3540-5. It is therefore an object of the invention to identify proteins and nucleic acids related to TNFs. Such proteins are believed to play a role in inflammatory and immune processes, suggesting their usefulness in treating autoimmune and inflammatory disorders.

A number of research groups have recently identified TNF family ligands with the same or substantially similar sequence, but they have not identified the associated receptor. The ligand has been variously named neutrokine α (WO 98/18921, published May 7, 1998), 63954 (WO 98/27114, published June 25, 1998), TL5 (EP 869 180, published October 7, 1998), NTN-2 (WO 98/55620 and WO 98/55621, published December 10, 1998), TNRL1-alpha (WO 9911791, published March 11, 1999), kay ligand (WO99/12964, published March 18, 1999), and AGP-3 (U.S. Prov. App. Nos. 60/119,906, filed February 12, 1999 and 60/166,271, filed November 18, 1999, respectively). Each of these references is hereby incorporated by reference. A need exists in the art for a receptor binding to the ligands described in these references.

In unrelated research, Bram and von Bulow discovered a lymphocyte surface receptor named Transmembrane Activator and CAML Interactor (TACI) protein. See WO 98/39361, published September 11, 1998, and von Bulow & Bram (1997), Science 278: 138-140, which are hereby incorporated by reference. According to these references, TACI binds an intracellular cyclophilin ligand designated CAML, which modulates the calcium signaling pathway in lymphocytes.

Summary of the Invention

In accordance with the present invention, the inventors describe a receptor for neutrokine α, 63954, TL5, NTN-2, TNRL1-alpha, kay ligand,

and AGP-3. The novel TNF ligand family member is herein called AGP-3 or TBAF (TNF family B cell Activation Factor) and its receptor is herein named AGP-3 R-or TBAF R. Unlike other members of the family, the receptor for AGP-3 is primarily expressed in B cells, and its expression correlates to increases in the number of B cells and immunoglobulins produced.

The natural, preferred human ortholog of the receptor is here called hAGP-3Rand contains 273 amino acids. The AGP-3 R-protein is a type III transmembrane protein and has an N- terminal extracellular domain, a transmembrane domain, and a C- terminal intracellular domain.

The AGP-3-R-related proteins of the invention may be membrane-associated or in soluble form, recombinantly produced or isolated after natural production. Such proteins are useful for the treatment of autoimmune or inflammatory conditions, particularly B-cell related autoimmune or inflammatory conditions. AGP-3-R-related proteins comprising the extracellular domain of AGP-3 R, as well as antibodies to AGP-3R, are preferred for treatment of B-cell-related autoimmune or inflammatory conditions. A most preferred indication for AGP-3-R-related proteins and antibodies is lupus.

The present specification also describes nucleic acids encoding AGP-3 R-related proteins, vectors and host cells expressing the polypeptides, and methods for producing recombinant proteins. Antibodies or fragments thereof that specifically bind AGP-3 R-are also provided.

The subject proteins may be used in assays to identify cells and tissues that express AGP-3 R-or proteins related to AGP-3-R, and to identify new AGP-3 R-related proteins. Methods of identifying compounds that interact with AGP-3 R-proteins are also provided. Such compounds include nucleic acids, peptides, proteins, carbohydrates, lipids

or small molecular weight organic molecules and may act either as agonists or antagonists of AGP-3 R-protein activity.

AGP-3 R related proteins are involved in B cell growth, survival, and activation, particularly in the lymph node, spleen, and Peyer's patches. AGP-3R agonists and antagonists (e.g., molecules incorporating the preferred regions of AGP-3 described below) thus modulate B cell response and may be used to treat diseases characterized by inflammatory processes or deregulated immune response, such as RA, GvHD, CD, lupus, and the like. Methods of use and pharmaceutical compositions comprising AGP-3 R-related proteins and AGP-3R agonists and antagonists are also encompassed by the invention.

In addition to therapeutic applications, AGP-3R related proteins may also be useful in production of hybridoma cells, which are derived from B cells. Thus, the present invention also concerns a method for modulating hybridoma cell antibody production, which comprises treating hybridoma cells with AGP-3R-related proteins.

Description of the Figures

Figure 1 shows the sequence of human AGP-3. Nucleic acid and amino acid sequences of human AGP-3 are indicated (SEQ ID NOS: 1 and 2, respectively). The predicted transmembrane region is underlined. Potential N-linked glycosylation sites are shown in boldface.

Figure 2 shows the sequence of murine AGP-3. Nucleic acid and amino acid sequences of murine AGP-3 are indicated (SEQ ID NOS: 3 and 4, respectively). The predicted transmembrane region is underlined. Potential N-linked glycosylation sites are shown in boldface.

Figure 3 shows an alignment of human and murine AGP-3, along with a consensus sequence (SEQ ID NO: 5). The predicted human and murine AGP-3 protein sequences were aligned by Pileup with gap creation penalty (12) and gap extension penalty (4) (Wisconsin GCG

Package, Version 8.1, Genetics Computer Group Inc., Madison, Wisconsin). The consensus sequence was determined by Lineup (Wisconsin GCG Package, Version 8.1). The transmembrane regions from amino acid 47 to 72 in human AGP-3 and from amino acid 48 to 73 in murine AGP-3 are underlined. The N-terminal intracellular domain resides from amino acid 1 to 46 in human AGP-3 and from amino acid 1 to 47 in murine AGP-3. The C-terminal extracellular domain is localized from amino acid 73 to 285 in human AGP-3, and from amino acid 74 to 309. The human and murine AGP-3 share 68% amino acid identity overall. The C-terminus of AGP-3 is more conserved between human and mouse, with 87% identity over a 142-amino acid length. The putative conserved beta strands are indicated at the top, with the amino acids forming the putative strands underlined.

Figure 4 shows human and murine AGP-3 mRNA tissue distribution. Human tissue northern blots (A) and murine tissue northern blots (B) were probed with ^{32}P-labeled human AGP-3 probe (A) or murine AGP-3 probe. The probed blots were exposed to Kodak film for 18 hours (A) or seven days (B).

Figure 5 shows histology analysis of AGP-3 transgenic mouse spleen. The spleen sections from control mouse (A, C and E) and AGP-3 transgenic mouse (B, D, and F) were stained with hematoxylin and exosin (A and B), anti-mouse B220 (C and D) or anti-mouse CD3 (E and F). The spleen of the transgenic mouse was enlarged, mainly due to the increase of size and number of the follicles. The B cell staining areas in the spleen follicles in the transgenic mouse were enlarged. The T cell number was slightly diminished.

Figure 6 shows histology analysis of AGP-3 transgenic mouse lymph nodes. The lymph node sections from control mouse (A, C and E) and AGP-3 transgenic mouse (B, D, and F) were stained with hematoxylin

and exosin (A and B), anti-mouse B220 (C and D) or anti-mouse CD3 (E and F). The lymph node size of the transgenic mouse was enlarged. The B cell number was greatly increased in the transgenic mouse. Instead of restricted to marginal zones of the follicles as in the control mouse, the B cells also filled out the follicular area in the lymph nodes of the transgenic mouse. The T cell number was decreased in the transgenic mouse as compared to the control.

Figure 7 shows histology analysis of AGP-3 transgenic mouse Peyer's patches. The Peyer's patches sections from control mouse (A, C and E) and AGP-3 transgenic mouse (B, D, and F) were stained with hematoxylin and exosin (A and B), anti-mouse B220 (C and D) or anti-mouse CD3 (E and F). The histologic and immunohistologic changes were similar to the changes in the lymph node of the transgenic mouse.

Figure 8 shows FACS analysis of thymocytes, splenocytes and lymph node cells from AGP-3 transgenic mouse. Single-cell suspensions were prepared from spleen, lymph nodes and thymus from 10 AGP-3 transgenic mice and 5 control littermates. Cells were stained with FITC or PE-conjugated monoclonal antibodies against Thy-1.2, B220, CD11b, Gr-1, CD4 or CD8. The B cell population increased by 100% in the transgenic mice as compared to the control mice. The T cell population decreased approximately 36%, with similar reductions in both CD4+ and CD8+ populations. Similar changes, though to a lesser degree, were observed in splenocytes. No differences in thymocyte staining were observed between the transgenic or control group.

Figure 9 shows a sequence comparison of the C-terminal region of members of the TNF ligand family determined via Pileup (Wisconsin GCG Package, Version 8.1). Amino acid numbers are indicated on the left side. The putative conserved beta strands and loops are indicated at the top. The predicted N-glycosylation sites are indicated with asterisks. The top

line shows the consensus sequence (SEQ ID NO: 6). The remaining lines show the sequence for the C-terminal region of the mammalian TNF-related protein identified (SEQ ID NOS: 7 to 24, 40).

Figure 10 shows histology analysis of AGP-3 transgenic mice. Sections of spleen (A, B, C), lymph node (D, E, F) and Payer's patches (G, H, I) from control mice (left panel)and AGP-3 transgenic mice (right panel) were stained with hematoxylin and exosin (A, D, and G), anti-mouse B220 antibody (B, E, and H), or anti-mouse CD3 antibody (C, F, and I). Stained sections were analyzed under microscope at 10x.

Figure 11 shows FACS analysis splenocytes, lymph node cells and thymocytes of AGP-3 transgenic mice. Single-cell suspensions were prepared form spleen, lymph nodes and thymus from 10 AGP-3 transgenic mice and 5 control littermates. Cells with stained with FITC or PE-conjugated monoclonal antibodies against thy-1.2, B220, CD11b, Gr-1, CD4 or CD8.

Figure 12 shows elevation of serum immunoglobulin levels in AGP-3 transgenic mice. Control mice (n=5) and AGP-3 transgenic mice (n=5) were bled successively at 6, 7, 8, 9, 11 and 12 weeks of age. Serum IgM, IgG, IgA, and IgE levels were quantitated by ELISA. Values are expressed as Mean ± SEM. All AGP-3 immunoglobulin levels were significantly increased (T-test; $P < 0.05$) compared to control groups.

Figure 13 shows kidney immunoglobulin deposits in AGP-3 transgenic mice. Kidney sections of 5 month control littermate (A, B, C), 5 month old AGP-3 mice (D, E, F), and 8 month old AGP-3 mice (G, H, I) were stained hematoxylin and exosin (A, D, and G), anti-mouse IgM (B, E, and H), anti-mouse IgG (C, F, and I), and Trichrome (G insert) Stained sections were analyzed under microscope at 60x.

Figure 14 shows that AGP-3 stimulates B cell survival and proliferation.

A. Increased B cell viability in AGP-3 transgenic mice. B cells were isolated from spleens of 3 month old AGP-3 transgenic mice (n-3) and control littermates (n=3). A total of $2.5x10^5$ B cells was aliquoted per well in a 96-well round bottom plate and incubated for 9 days. At the indicated days, cells were incubated with 5 •g/ml Propidium Iodide and subject to FACS analysis for positive staining cells. Values are expressed as Mean ± SEM.

B. AGP-3 stimulates B cell proliferation. Purified B cells (10^5) from B6 mice were cultured in triplicates in 96 well plate with indicated amount of AGP-3 at the absence (upper panel) or presence of 2 •g/ml anti-IgM antibody (lower panel) for a period of 4 days. Proliferation was measured by radioactive ^{3}(H) thymidine uptake in last 18 hours of pulse. Data shown represent mean ± standard deviation of triplicate wells.

Figure 15 shows identification of AGP-3 receptor source. Approximately 10^6 cells of each type were exposed to 1 μg/ml Flag-AGP-3 protein in the absence or presence of 10 μg/ml AGP-3 protein as specific competitor. Cells were subsequently incubated with 20 μg/ml anti-FLAG M2 monoclonal antibody, and then with 20 μg/ml FITC-conjugated goat anti-mouse IgG. Cells were finally analyzed by fluorescence activated cell sorting (FACS) using a Becton Dickinson FACscan.

Figure 16 shows alignment of AGP-3 binding clones RAJI-13B4 and 13H11. The cDNA insert sequences from two positive binding clones 13B4 and 13H11 were aligned by GAP with gap creation penalty (12) and gap extension penalty (4) (Wisconsin GCG Package, Version 8.1, Genetics Computer Group Inc., Madison, Wisconsin). Two positive clones encode the same gene, with extra 7 bp at the N-terminus of clone 13H11.

Figure 17 shows the nucleic acid and amino acid sequences (SEQ ID NOS: 41 and 42) of human AGP-3 receptor.

Figure 18 shows the protein sequence of human AGP-3 receptor. The extracellular domain (SEQ ID NO: 43) includes the N-terminal domain (top line shown in Figure 18, SEQ ID NO: 44) through two cysteine-rich repeats (labeled I and II, SEQ ID NOS: 45 and 46) to the end of the "stalk" region (SEQ ID NO: 47). The transmembrane domain (labeled TM, SEQ ID NO: 48) is underlined, and the intracellular domain (labeled IC, SEQ ID NO: 49) is also indicated.

Figure 19. Alignment of extracellular domains of human AGP-3 receptor and TNFR1. Extracellular domain of human AGP-3 receptor and TNFR1 were aligned by GAP with gap creation penalty (12) and gap extension penalty (4) (Wisconsin GCG Package, Version 8.1, Genetics Computer Group Inc., Madison, Wisconsin).

Figure 20. Northern analysis of human AGP-3 receptor. Human tissue northern blots were probed with ^{32}P-labeled human AGP-3 receptor probe. The probed blots were exposed to Kodak film for 18 hours.

Figure 21. Extracellular domain of AGP-3R binds to AGP-3.

A. Western analysis of AGP-3R-Fc fusion protein. 293 cells were transfected with control vector or AGP-3R-Fc/pCEP4 expression vector which directs synthesis extracellular domain of AGP-3R fused human IgG Fc at the C-terminus. After 24 hour transfection, medium and cell lysates were subject to western analysis with anti-Fc antibody. The AGP-3R-Fc fusion protein was detected only in the transfected cell lysates, not in the medium. This supports that AGP-3 receptor extracellular domain lacks a N-terminal signal peptide.

B. AGP-3R-Fc fusion protein binds AGP-3. Cell lysates that contain AGP-3R-Fc fusion protein generated as described above were incubated with FLAG-AGP-3 protein and protein A beads for 1 hr at 4°C. The protein A beads were washed with E1A buffer for 6 times. The precipitates were fractionated by SDS-PAGE and subject to western

blot analysis by anti-FLAG antibody. FLAG-AGP-3 was co-precipitated by AGP-3R-Fc cell lysates.

AGP-3Detailed Description of the Invention

Definition of Terms

The following definitions apply to the terms used throughout this specification, unless otherwise limited in specific instances.

The term "AGP-3 related protein" refers to natural and recombinant proteins comprising the following sequence:

QDCLQLIADSXTPTIXKGXYTFVPWLLSF

(SEQ ID NO: 25)

wherein "X" may be any naturally occurring amino acid residue. This sequence is a consensus of the B and B′ β-sheets and B/B′ loop of hAGP-3 and mAGP-3 (see Figure 3), which is believed to be the specific receptor binding site. Preferred AGP-3-related proteins comprise both the B/B′ consensus and the E/F consensus:

AMGHXIQRKKVHVFGDELSLVTLFR

(SEQ ID NO: 26)

The E/F region is also believed to be involved in receptor binding. More preferred proteins are those comprising the consensus of the B-I region:

QDCLQLIADS XTPTIXKGXY TFVPWLLSFK RGXALEEKEN KIXVXXTGYF FIYXQVLYTD XXXAMGHXIQ RKKVHVFGDE LSLVTLFRCI QNMPXTLPNN SCYSAGIAXL EEGDEXQLAI PRENAQISXX GDXTFFGALK LL

(SEQ ID NO: 27)

"AGP-3-related activity" means that a natural or recombinant protein (including antibodies), analog, derivative or fragment that (a) is capable of interacting with an AGP-3-related protein or (b) has the same binding site on an AGP-3R-related protein as an AGP-3-related protein, and thereby is capable of modulating B cell growth, survival, or activation. Of particular interest is such AGP-3-related activity in MLN, spleen, and

Peyer's patches. The inventors contemplate that some molecules of interest may have activity antagonistic to native AGP-3 activity; for example, a derivative or analog may retain AGP-3 binding activity but will not activate the AGP-3 receptor. All such activity (agonism and antagonism of AGP-3) falls within the meaning of "AGP-3 related activity." Such activity can be determined, for example, by such assays as described in "Biological activity of AGP-3" in the Materials & Methods hereinafter, which may be modified as needed by many methods known to persons having ordinary skill in the art.

The term "AGP-3R related protein" refers to proteins comprising the cystein-rich repeats (SEQ ID NOS: 45 and 46) of the extracellular domain (SEQ ID NO: 43) of AGP-3-R. Such proteins having at least about 80% identity with the extracellular domain are preferred, with those having 90% or 95% identity or greater more preferred. Most preferred proteins comprise the sequence or sequences of the amino acids that interact with the B/B' and/or E/F regions of AGP-3, or more generally with the B-I region of AGP-3. Such sequences can be included in naturally occurring proteins, truncated naturally occurring proteins, or recombinant and synthetic proteins. Recombinant and synthetic AGP-3R-related proteins may be formed by fusion of the AGP-3R-derived fragment with unrelated molecules or molecular domains (e.g., Fc regions), domain swapping with other TNF receptor family members, antibody grafting (e.g., substituting an AGP-3R fragment sequence for an antibody CDR or variable domain), or other modifications. Such proteins are discussed further hereinbelow. The proteins may also be modified by linkage to a carbohydrate (e.g., dextran) or a water-soluble polymer (e.g., PEG). The proteins within this definition may also include substitution with amino acids serving as sites for attachment of non-protein groups (e.g.,

glycosylation sites). All such proteins are encompassed by the terms "AGP-3R related protein."

An "analog" of an AGP-3R protein (e.g., hAGP-3R) is a polypeptide within the definition of "AGP-3R-related protein" that has a substitution or addition of one or more amino acids. Such an AGP-3R-related protein should maintain the property of eliciting B cell growth, survival, or activation. Such analogs will have substitutions or additions at any place along the polypeptide. Preferred analogs include those of soluble AGP-3R-related proteins. Fragments or analogs may be naturally occurring, such as a polypeptide product of an allelic variant or a mRNA splice variant, or they may be constructed using techniques available to one skilled in the art for manipulating and synthesizing nucleic acids. The polypeptides may or may not have an amino terminal methionine residue.

A "derivative" of an AGP-3R-protein is a polypeptide within the definition of "AGP-3R-related protein" that has undergone post-translational modifications. Such modifications include, for example, addition of N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends, attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition of an N-terminal methionine residue due to prokaryotic host cell expression. In particular, chemically modified derivatives of AGP-3R-related protein that provide additional advantages such as increased stability, longer circulating time, or decreased immunogenicity are contemplated. Of particular use is modification with water soluble polymers, such as polyethylene glycol and derivatives thereof (see for example U.S. Patent No. 4,179,337). The chemical moieties for derivitization may be selected from water-soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The

polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties. Polypeptides may also be modified at pre-determined positions in the polypeptide, such as at the amino terminus, or at a selected lysine or arginine residue within the polypeptide. Other chemical modifications provided include a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

The term "protein" refers to polypeptides regardless of length or origin, comprising molecules that are recombinantly produced or naturally occurring, full length or truncated, having a natural sequence or mutated sequence, with or without post-translational modification, whether produced in mammalian cells, bacterial cells, or any other expression system.

Ligand for the receptor

The natural, preferred human ortholog of the associated ligand (hAGP-3) contains 285 amino acids; the mouse ortholog (mAGP-3), contains 309 amino acids. The AGP-3 protein is a type II transmembrane protein and has an amino terminal cytoplasmic domain, a transmembrane domain, and a carboxy terminal extracellular domain. TNF-related proteins of the invention may be membrane-associated or in soluble form, recombinantly produced or isolated after natural production. The present specification demonstrates that AGP-3 is a potent B cell stimulatory factor. Interestingly, the AGP-3 transgenic mice also developed autoantibodies and kidney immune complex deposits, a phenotype resembling lupus patients and lupus prone mice.

AGP-3-related protein primarily acts on B cells. An EST bearing a portion of the AGP-3 sequence was obtained from a human fetal liver spleen cDNA library. A labeled cDNA fragment was used to probe a

human spleen cDNA phage library (see "Cloning of Human AGP-3" in Materials & Methods hereinafter). The cDNA encoding a human AGP-3 was isolated from this phage library. The human protein is a type II transmembrane protein, having a short N-terminal intracellular region that differed from other members of the TNF ligand family and a long C-terminal extracellular region that comprises most of the conserved region of the TNF ligand family.

An EST encoding a murine ortholog of AGP-3 was identified by BLAST search of Genebank using the human AGP-3 sequence. The corresponding cDNA clone was obtained from a mouse lymph node library and used to probe a mouse spleen cDNA phage library (see Materials & Methods hereinafter). The cDNA encoding a murine AGP-3 ortholog was isolated from this phage library.

Northern blots were used to determine tissue distribution of transcription of AGP-3 (see "Cloning of Murine AGP-3" in Materials & Methods hereinafter). In murine tissue, AGP-3 mRNA was detected mainly in spleen, lung, liver, and kidney. In human tissue, AGP-3 mRNA was detected predominantly in peripheral blood leukocytes, with weaker transcription in spleen, lung, and small intestine (see Figures 4A and 4B).

The murine ortholog of AGP-3 was overexpressed in transgenic mice (see "Overexpression of murine AGP-3 in transgenic mice" in Materials & Methods hereinafter). In these transgenic mice, serum globulin and total protein levels increased greatly over control littermates while the albumin level remained the same (see "Biological Activity of AGP-3" in Materials & Methods hereinafter). The mice also exhibited increases in the size and number of follicles in the spleen, lymph nodes, and Peyer's patches (Figures 5, 6, and 7). In their MLN, the mice exhibited 100% increases in the number of cells expressing CD45 receptor with concomitant decreases in cells expressing CD90, CD4, and CD8. These

results correspond to an increase in the B cell population and a decrease in the T cell population in the MLN (Figures 6 and 8). Similar results were obtained in the spleen, but to a lesser extent (Figures 5 and 8).

Nucleic Acids

The invention provides for isolated nucleic acids encoding AGP-3 R-related proteins. As used herein, the term nucleic acid comprises cDNA, genomic DNA, wholly or partially synthetic DNA, and RNA. These nucleic acids may be prepared or isolated as described in the working examples hereinafter or by nucleic acid hybridization thereof.

Nucleic acid hybridization typically involves a multi-step process. A first hybridization step forms nucleic acid duplexes from single strands. A second hybridization step under more stringent conditions selectively retains nucleic acid duplexes having the desired homology. The conditions of the first hybridization step are generally not crucial, provided they are not of higher stringency than the second hybridization step. Generally, the second hybridization is carried out under conditions of high stringency, wherein "high stringency" conditions refers to conditions of temperature and salt that are about 12-20 °C below the melting temperature (T_m) of a perfect hybrid of part or all of the complementary strand corresponding to the AGP-3R extracellular domain shown in Figure 17. In one embodiment, "high stringency" conditions refer to conditions of about 65 °C and not more than about 1 M Na^+. It is understood that salt concentration, temperature and/or length of incubation may be varied in either the first or second hybridization steps such that one obtains the hybridizing nucleic acid molecules according to the invention. Conditions for hybridization of nucleic acids and calculations of T_m for nucleic acid hybrids are described in Sambrook et al. (1989), Molecular Cloning: A Laboratory Manual Cold Spring Harbor Laboratory Press, New York.

The nucleic acids of the invention may hybridize to part or all of the polypeptide coding regions of AGP-3R related proteins (e.g., as shown in Figure 17) and therefore may be truncations or extensions of the nucleic acid sequences shown therein. Truncated or extended nucleic acids are encompassed by the invention provided that the encoded proteins retain AGP-3 related activity. In one embodiment, the nucleic acid will encode a polypeptide of at least about 10 amino acids. In another embodiment, the nucleic acid will encode a polypeptide of at least about 20 amino acids. In yet another embodiment, the nucleic acid will encode a polypeptide of at least about 50 amino acids. The hybridizing nucleic acids may also include noncoding sequences located 5′ and/or 3′ to the coding regions for the AGP-3R related protein. Noncoding sequences include regulatory regions involved in expression of AGP-3R-related protein, such as promoters, enhancer regions, translational initiation sites, transcription termination sites and the like.

In preferred embodiments, the nucleic acids of the invention encode human AGP-3-R. Most preferred are the nucleic acids encoding the extracellular domain. For molecules encoding AGP-3R comprising the transmembrane domain, substitutions that replace hydrophobic amino acid residues in this region with neutral or hydrophilic amino acid residues would be expected to disrupt membrane association and result in soluble AGP-3R-related protein. In addition, deletions of part or all the transmembrane region would also be expected to produce soluble forms of AGP-3R-related protein.

Nucleic acid sequences of the invention may also be used for the detection of sequences encoding AGP-3R-related protein in biological samples. In particular, the sequences may be used to screen cDNA and genomic libraries for AGP-3R-related protein sequences, especially those from other species. The nucleic acids are also useful for modulating levels

of AGP-3 R-related protein by anti-sense technology or in vivo gene expression. Development of transgenic animals expressing AGP-3R-related protein are useful for production of the polypeptides and for the study of in vivo biological activity.

Vectors and Host Cells

The nucleic acids of the invention will be linked with DNA sequences so as to express biologically active AGP-3 R-related protein. Sequences required for expression are known to those skilled in the art and include promoters and enhancer sequences for initiation of RNA synthesis, transcription termination sites, ribosome binding sites for the initiation of protein synthesis, and leader sequences for secretion. Sequences directing expression and secretion of AGP-3 R-related protein may be homologous, i.e., the sequences are identical or similar to those sequences in the genome involved in AGP-3 R-related protein expression and secretion, or they may be heterologous. A variety of plasmid vectors are available for expressing AGP-3R-related protein in host cells; see, for example, Methods in Enzymology v. 185, Goeddel, D.V. ed., Academic Press (1990). For expression in mammalian host cells, a preferred embodiment is plasmid pDSRα described in PCT Application No. 90/14363. For expression in bacterial host cells, preferred embodiments include plasmids harboring the lux promoter (see co-owned and co-pending U.S. Serial No. 08/577,778, filed December 22, 1995). In addition, vectors are available for the tissue-specific expression of AGP-3 R-related protein in transgenic animals. Gene transfer vectors derived from retrovirus (RV), adenovirus (AdV), and adeno-associated virus (AAV) may also be used for the expression of AGP-3R-related protein in human cells for in vivo therapy (see PCT Application No. 86/00922).

Prokaryotic and eukaryotic host cells expressing AGP-3 R-related protein are also provided by the invention. Host cells include bacterial,

yeast, plant, insect or mammalian cells. AGP-3 R-related protein may also be produced in transgenic animals, such as mice or goats. Plasmids and vectors containing the nucleic acids of the invention are introduced into appropriate host cells using transfection or transformation techniques known to one skilled in the art. Host cells may contain DNA sequences encoding AGP-3 R-related protein as shown in Figure 17 or a portion thereof, such as the extracellular domain or the cytoplasmic domain. Nucleic acids encoding AGP-3 R-related proteins may be modified by substitution of codons that allow for optimal expression in a given host. At least some of the codons may be so-called preference codons that do not alter the amino acid sequence and are frequently found in genes that are highly expressed. However, it is understood that codon alterations to optimize expression are not restricted to the introduction of preference codons. Examples of preferred mammalian host cells for AGP-3 R-related protein expression include, but are not limited to COS, CHOd-, 293 and 3T3 cells. A preferred bacterial host cell is Escherichia coli.

Polypeptides

The invention also provides AGP-3 R-related protein as the products of prokaryotic or eukaryotic expression of an exogenous DNA sequences. Exogenous DNA sequences include cDNA, genomic DNA and synthetic DNA sequences. AGP-3 R-related proteins may be the products of bacterial, yeast, plant, insect or mammalian cells expression, or from cell-free translation systems. AGP-3 R-related proteins produced in bacterial cells will have N-terminal methionine residues. The invention also provides for a process of producing AGP-3 R-related proteins comprising growing prokaryotic or eukaryotic host cells transformed or transfected with nucleic acids encoding them and isolating polypeptide expression products of the nucleic acids.

Polypeptides that are mammalian proteins or are fragments, analogs or derivatives thereof are encompassed by the invention. In preferred embodiments, the AGP-3R-related protein is human AGP-3 R. A fragment of AGP-3R-related protein refers to a polypeptide having a deletion of one or more amino acids such that the resulting polypeptide retains AGP-3 related activity; for example, the polypeptide has at least the property of antagonizing B cell growth, survival, or activation, especially in mesenteric lymph nodes. Said fragments will have deletions originating from the amino terminal end, the carboxy terminal end, or internal regions of the polypeptide. Fragments of AGP-3 R-related proteins are at least about ten amino acids, at least about 20 amino acids, or at least about 50 amino acids in length. In preferred embodiments, AGP-3 R-related proteins will have a deletion of one or more amino acids from the transmembrane region (see Figure 17), or, alternatively, one or more amino acids from the amino-terminus up to and/or including the transmembrane region.

The polypeptides of the invention are isolated and purified from tissues and cell lines that express AGP-3 R-related protein, either extracted from lysates or from conditioned growth medium, and from transformed host cells expressing AGP-3 R-related protein. Human AGP-3 R-related protein, or nucleic acids encoding same, may be isolated from human lymph node or fetal liver tissue. Isolated AGP-3 R-related protein is free from association with human proteins and other cell constituents.

A method for purification of such proteins from natural sources (e.g. tissues and cell lines that normally express an AGP-3R related protein) and from transfected host cells is also encompassed by the invention. The purification process may employ one or more standard protein purification steps in an appropriate order to obtain purified protein. The chromatography steps can include ion exchange, gel

filtration, hydrophobic interaction, reverse phase, chromatofocusing, affinity chromatography employing an anti-AGP-3 R-related protein antibody or biotin-streptavidin affinity complex and the like.

Fusion proteins and derivatives

The invention further comprises AGP-3 R-related protein chimeras, as well as such proteins derivatized by linkage to such molecules as PEG or dextran. Such proteins comprise part or all of an AGP-3 R-related protein amino acid sequence fused to a heterologous amino acid sequence. The heterologous sequence may be any sequence that allows the resulting fusion protein to retain AGP-3 R-related activity. In preferred embodiments, a heterologous sequence is fused to a portion of an AGP-3 R-related protein's sequence that interacts with an AGP-3 related protein's B/B' region (SEQ ID NO: 25) and/or the E/F region (SEQ ID NO: 26) or with the more complete B-I region (SEQ ID NO: 27). Such heterologous sequences include cytoplasmic domains that allow for alternative intracellular signaling events, sequences that promote oligomerization (e.g., the Fc region of IgG), enzyme sequences that provide a label for the polypeptide, and sequences that provide affinity probes (e.g., an antigen-antibody recognition site).

Preferred molecules in accordance with this invention are Fc-linked AGP-3 R-related proteins. Useful modifications of protein therapeutic agents by fusion with the "Fc" domain of an antibody are discussed in detail in a patent application entitled, "Modified Peptides as Therapeutic Agents," U.S. Ser. No. 09/428,082, PCT appl. no. WO 99/25044, which is hereby incorporated by reference in its entirety. That patent application discusses linkage to a "vehicle" such as PEG, dextran, or an Fc region.

In the compositions of matter prepared in accordance with this invention, the AGP-3 R-related protein may be attached to a vehicle

through the protein's N-terminus or C-terminus. Thus, the vehicle-protein molecules of this invention may be described by the following formula I:

I

$$(X^1)_a\text{-}F^1\text{-}(X^2)_b$$

wherein:

F^1 is a vehicle (preferably an Fc domain);

X^1 and X^2 are each independently selected from $-(L^1)_c\text{-}P^1$, $-(L^1)_c\text{-}P^1\text{-}(L^2)_d\text{-}P^2$, $-(L^1)_c\text{-}P^1\text{-}(L^2)_d\text{-}P^2\text{-}(L^3)_e\text{-}P^3$, and $-(L^1)_c\text{-}P^1\text{-}(L^2)_d\text{-}P^2\text{-}(L^3)_e\text{-}P^3\text{-}(L^4)_f\text{-}P^4$

P^1, P^2, P^3, and P^4 are each independently sequences of an AGP-3 R-related protein (e.g., a fragment of hAGP-3R that is capable of binding to AGP-3) and are preferably selected from SEQ ID NO: 38, 39, 40, and 41;

L^1, L^2, L^3, and L^4 are each independently linkers; and

a, b, c, d, e, and f are each independently 0 or 1, provided that at least one of a and b is 1.

Thus, compound I comprises preferred compounds of the formulae

II

$$X^1\text{-}F^1$$

and multimers thereof wherein F^1 is an Fc domain and is attached at the C-terminus of X^1;

III

$$F^1\text{-}X^2$$

and multimers thereof wherein F^1 is an Fc domain and is attached at the N-terminus of X^2;

IV

$$F^1\text{-}(L^1)_c\text{-}P^1$$

and multimers thereof wherein F^1 is an Fc domain and is attached at the N-terminus of $-(L^1)_c\text{-}P^1$; and

V

$$F^1\text{-}(L^1)_c\text{-}P^1\text{-}(L^2)_d\text{-}P^2$$

and multimers thereof wherein F^1 is an Fc domain and is attached at the N-terminus of $-L^1-P^1-L^2-P^2$.

Antibodies

Uses for antibodies specifically binding the polypeptides of the invention are also encompassed by the invention. The antibodies may be generated by immunization with full-length AGP-3R related protein, or fragments thereof. Preferred antibodies bind to the portions of AGP-3R that interact with the B/B' and/or E/F regions of AGP-3 or more generally with the B-I region. Such antibodies may be generated by immunization with polypeptides comprising those portions of AGP-3R. The term "antibodies" also refers to molecules having Fv, Fc and other structural domains usually associated with antibodies but that may be generated by other techniques (e.g., phage display antibody generation). The antibodies of the invention may be polyclonal or monoclonal, or may be recombinant antibodies, such as chimeric antibodies wherein the murine constant regions on light and heavy chains are replaced by human sequences, or CDR-grafted antibodies wherein only the complementarity determining regions are of murine origin. Antibodies of the invention may also be fully human antibodies prepared, for example, by immunization of transgenic animals capable of producing human antibodies (see, for example, PCT Application No. WO93/12227). Regardless of the means by which they are generated, antibodies in accordance with this invention may be produced by recombinant means (e.g., transfection of CHO cells with vectors comprising antibody sequence).

The antibodies are useful for detecting AGP-3R-related protein in biological samples, thereby allowing the identification of cells or tissues that produce such proteins. In addition, antibodies that bind to AGP-3R related proteins and block interaction with other binding compounds (i.e., "antagonist antibodies") have therapeutic use in modulating B cell

growth, activation, and/or proliferation. On the other hand, antibodies that bind to AGP-3R and activate the receptor as would AGP-3 ("agonist antibodies") have therapeutic use in conditions in which the patient would benefit from B cell growth, activation or proliferation (e.g., in patients immunocompromised due to chemotherapy or acquired immune deficiency syndrome). Antibodies can be tested for binding to AGP-3R related protein and examined for their effect on AGP-3-mediated B cell growth, survival, or activation associated with the disease or condition (see "Biological activity of AGP-3" in Materials & Methods hereinafter).

Compositions

The invention also provides for pharmaceutical compositions comprising a therapeutically effective amount of the AGP-3 related protein or AGP-3 R-related protein of the invention together with a pharmaceutically acceptable diluent, carrier, solubilizer, emulsifier, preservative and/or adjuvant. The invention also provides for pharmaceutical compositions comprising a therapeutically effective amount of an AGP-3 related or AGP-3 R-related protein agonist or antagonist. The term "therapeutically effective amount" means an amount that provides a therapeutic effect for a specified condition and route of administration. The composition may be in a liquid or lyophilized form and comprises one or more of the following:

- a diluent (e.g., Tris, acetate or phosphate buffers) having various pH values and ionic strengths;
- a solubilizer (e.g., Tween or Polysorbate);
- carriers (e.g., human serum albumin or gelatin);
- preservatives (e.g., thimerosal or benzyl alcohol); and
- antioxidants (e.g., ascorbic acid or sodium metabisulfite).

Selection of a particular composition will depend upon a number of factors, including the condition being treated, the route of administration

and the pharmacokinetic parameters desired. A more extensive survey of component suitable for pharmaceutical compositions is found in Remington's Pharmaceutical Sciences (1980), 18th ed. (A. R. Gennaro, ed.) Mack, Easton, PA.

In a preferred embodiment, compositions comprising soluble AGP-3 R-related proteins are provided. Also encompassed are compositions comprising soluble AGP-3R-related protein modified with water-soluble polymers to increase solubility, stability, plasma half-life and bioavailability. Compositions may also comprise incorporation of soluble AGP-3 R-related protein into liposomes, microemulsions, micelles or vesicles for controlled delivery over an extended period of time. Soluble AGP-3 R-related protein may be formulated into microparticles suitable for pulmonary administration.

Compositions of the invention may be administered by injection (either subcutaneous, intravenous or intramuscular) or by oral, nasal, pulmonary or rectal administration. The route of administration eventually chosen will depend upon a number of factors and may be ascertained by one of ordinary skill in the art.

The invention also provides for pharmaceutical compositions comprising a therapeutically effective amount of the nucleic acids of the invention together with a pharmaceutically acceptable adjuvant. Nucleic acid compositions will be suitable for the delivery of part or all of the coding region of AGP-3R-related protein and/or flanking regions to cells and tissues as part of an anti-sense therapy regimen.

Pharmaceutical Methods of Use

AGP-3R-related proteins and agonists or antagonists thereof may be used to treat conditions characterized by B cell growth, survival, and activation, such as autoimmune and inflammatory disorders. The invention also encompasses modulators (agonists and antagonists) of

AGP-3R-related protein and the methods for obtaining them. Such a modulator may either increase or decrease at least one form of AGP-3 related activity, such as B cell growth, survival, or activation in MLN, spleen, and Peyer's patches. Typically, an agonist or antagonist may be a co-factor, such as a protein, peptide, carbohydrate, lipid or small molecular weight molecule, that interacts with AGP-3R and regulates activity. Potential polypeptide antagonists include antibodies that react with AGP-3R, a soluble form of AGP-3R, fusion proteins comprising a soluble form of AGP-3R, and derivatives of soluble AGP-3R. Molecules that regulate AGP-3R-related protein expression typically include nucleic acids that are complementary to nucleic acids encoding AGP-3R-related protein or a fragment thereof and that act as anti-sense regulators of expression.

AGP-3R-related proteins and modulators thereof may be particularly useful in treatment of inflammatory conditions of the joints. Inflammatory conditions of a joint are chronic joint diseases that afflict and disable, to varying degrees, millions of people worldwide. Rheumatoid arthritis is a disease of articular joints in which the cartilage and bone are slowly eroded away by a proliferative, invasive connective tissue called pannus, which is derived from the synovial membrane. The disease may involve peri-articular structures such as bursae, tendon sheaths and tendons as well as extra-articular tissues such as the subcutis, cardiovascular system, lungs, spleen, lymph nodes, skeletal muscles, nervous system (central and peripheral) and eyes (Silberberg (1985), Anderson's Pathology, Kissane (ed.), II:1828). Osteoarthritis is a common joint disease characterized by degenerative changes in articular cartilage and reactive proliferation of bone and cartilage around the joint. Osteoarthritis is a cell-mediated active process that may result from the inappropriate response of chondrocytes to catabolic and anabolic stimuli.

Changes in some matrix molecules of articular cartilage reportedly occur in early osteoarthritis (Thonar et al. (1993), Rheumatic disease clinics of North America, Moskowitz (ed.), 19:635-657 and Shinmei et al. (1992), Arthritis Rheum., 35:1304-1308). AGP-3, AGP-3R and modulators thereof are believed to be useful in the treatment of these and related conditions.

AGP-3R-related proteins, and agonists or antagonists of either may also be useful in treatment of a number of additional diseases and disorders, including:

acute pancreatitis;

ALS;

Alzheimer's disease;

asthma;

atherosclerosis;

cachexia/anorexia;

chronic fatigue syndrome;

diabetes (e.g., insulin diabetes);

fever;

glomerulonephritis;

graft versus host disease;

hemorrhagic shock;

hyperalgesia;

inflammatory bowel disease;

inflammatory conditions of a joint, including osteoarthritis, psoriatic arthritis and rheumatoid arthritis;

inflammatory conditions resulting from strain, sprain, cartilage damage, trauma, orthopedic surgery, infection or other disease processes;

ischemic injury, including cerebral ischemia (e.g., brain injury as a result of trauma, epilepsy, hemorrhage or stroke, each of which may lead to neurodegeneration);

learning impairment;
lung diseases (e.g., ARDS);
multiple myeloma;
multiple sclerosis;
myelogenous (e.g., AML and CML) and other leukemias;
myopathies (e.g., muscle protein metabolism, esp. in sepsis);
neurotoxicity (e.g., as induced by HIV);
osteoporosis;
pain;
Parkinson's disease;
pre-term labor;
psoriasis;
reperfusion injury;
septic shock;
side effects from radiation therapy;
sleep disturbance;
temporal mandibular joint disease; and
tumor metastasis.

Agonists and antagonists of AGP-3R-related protein may be administered alone or in combination with a therapeutically effective amount of other drugs, including analgesic agents, disease-modifying anti-rheumatic drugs (DMARDs), non-steroidal anti-inflammatory drugs (NSAIDs), and any immune and/or inflammatory modulators. Thus, agonists and antagonists of AGP-3R-related protein may be administered with:

- Modulators of other members of the TNF/TNF receptor family, including TNF antagonists, such as etanercept (Enbrel™), sTNF-RI, D2E7, and Remicade™.
- Nerve growth factor (NGF) modulators.

- IL-1 inhibitors, including IL-1ra molecules such as anakinra (Kineret™) and more recently discovered IL-1ra-like molecules such as IL-1Hy1 and IL-1Hy2; IL-1 "trap" molecules as described in U.S. Pat. No. 5,844,099, issued December 1, 1998; IL-1 antibodies; solubilized IL-1 receptor, and the like.
- IL-6 inhibitors (e.g., antibodies to IL-6).
- IL-8 inhibitors (e.g., antibodies to IL-8).
- IL-18 inhibitors (e.g., IL-18 binding protein, solubilized IL-18 receptor, or IL-18 antibodies).
- Interleukin-1 converting enzyme (ICE) modulators.
- insulin-like growth factors (IGF-1, IGF-2) and modulators thereof.
- Transforming growth factor-β (TGF-β), TGF-β family members, and TGF-β modulators.
- Fibroblast growth factors FGF-1 to FGF-10, and FGF modulators.
- Osteoprotegerin (OPG), OPG analogues, osteoprotective agents, and bone anabolic agents.
- PAF antagonists.
- Keratinocyte growth factor (KGF), KGF-related molecules (e.g., KGF-2), and KGF modulators.
- COX-2 inhibitors, such as Celebrex™ and Vioxx™.
- Prostaglandin analogs (e.g., E series prostaglandins).
- Matrix metalloproteinase (MMP) modulators.
- Nitric oxide synthase (NOS) modulators, including modulators of inducible NOS.
- Modulators of glucocorticoid receptor.
- Modulators of glutamate receptor.
- Modulators of lipopolysaccharide (LPS) levels.

- Anti-cancer agents, including inhibitors of oncogenes (e.g., fos, jun) and interferons.
- Noradrenaline and modulators and mimetics thereof.

Assay Methods of Use

AGP-3 R-related proteins may be used in a variety of assays for detecting agonists, antagonists and characterizing interactions with AGP-3R-related proteins. In general, the assay comprises incubating AGP-3R-related protein under conditions that permit measurement of AGP-3-related activity as defined above. Qualitative or quantitative assays may be developed. Assays may also be used to identify new AGP-3R agonists or antagonists and AGP-3R protein family members.

Binding of natural or synthesized receptor, agonist, or antagonist to AGP-3R-related protein may be carried out in several formats, including cell-based binding assays, membrane binding assays, solution-phase assays and immunoassays. In general, trace levels of a labeled binding molecule are incubated with AGP-3R-related protein samples for a specified period of time followed by measurement of bound molecule by filtration, electrochemiluminescent (ECL, ORIGEN system by IGEN), cell-based or immunoassays. Homogeneous assay technologies for radioactivity (SPA; Amersham) and time-resolved fluoresence (HTRF, Packard) can also be implemented. Binding is detected by labeling a binding molecule (e.g., an anti-AGP-3R antibody) with radioactive isotopes (125I, 35S, 3H), fluorescent dyes (fluorescein), lanthanide (Eu^{3+}) chelates or cryptates, orbipyridyl-ruthenium (Ru^{2+}) complexes. It is understood that the choice of a labeled probe will depend upon the detection system used. Alternatively, a binding molecule may be modified with an unlabeled epitope tag (e.g., biotin, peptides, His_6, myc) and bound to proteins such as streptavidin, anti-peptide or anti-protein antibodies that have a detectable label as described above.

Binding molecules in such assays may be nucleic acids, proteins, peptides, carbohydrates, lipids or small molecular weight organic compounds. The binding molecule may be substantially purified or present in a crude mixture. The binding molecules may be further characterized by their ability to increase or decrease AGP-3-related activity in order to determine whether they act as an agonist or an antagonist.

In an alternative method, AGP-3 R-related protein may be assayed directly using polyclonal or monoclonal antibodies to AGP-3R-related proteins in an immunoassay. Additional forms of AGP-3R-related proteins containing epitope tags as described above may be used in solution and immunoassays.

AGP-3R-related proteins are also useful for identification of intracellular proteins that interact with their respective cytoplasmic domains by a yeast two-hybrid screening process. As an example, hybrid constructs comprising DNA encoding the N-terminal 50 amino acids of an AGP-3R-related protein fused to a yeast GAL4-DNA binding domain may be used as a two-hybrid bait plasmid. Positive clones emerging from the screening may be characterized further to identify interacting proteins. This information may help elucidate an intracellular signaling mechanism associated with AGP-3-related activity and provide intracellular targets for new drugs that modulate inflammatory and immune-related diseases and conditions.

A variety of assays may be used to measure the interaction of AGP-3R-related proteins and agonists, antagonists, or other ligands in vitro using purified proteins. These assays may be used to screen compounds for their ability to increase or decrease the rate or extent of binding to AGP-3R-related proteins. In one type of assay, AGP-3R-related protein can be immobilized by attachment to the bottom of the wells of a microtiter

plate. A radiolabeled binding molecule and a test molecule can then be added either one at a time (in either order) or simultaneously to the wells. After incubation, the wells can be washed and counted using a scintillation counter for radioactivity to determine the extent of binding to AGP-3R-related protein. Typically, molecules will be tested over a range of concentrations, and a series of control wells lacking one or more elements of the test assays can be used for accuracy in evaluation of the results. An alternative to this method involves reversing the "positions" of the proteins; i.e., immobilizing a binding molecule to the mictrotiter plate wells, incubating with the test compound and radiolabeled AGP-3 related protein, and determining the extent of binding. See, for example, chapter 18 of Current Protocols in Molecular Biology (1995) (Ausubel et al., eds.), John Wiley & Sons, New York, NY.

As an alternative to radiolabeling, AGP-3 R-related proteins or a binding molecule may be conjugated to biotin and the presence of biotinylated protein can then be detected using streptavidin linked to an enzyme, such as horseradish peroxidase (HRP) or alkaline phosphatase (AP), that can be detected colorimetrically, or by fluorescent tagging of streptavidin. An antibody directed to AGP-3R-related protein or a binding molecule that is conjugated to biotin may also be used and can be detected after incubation with enzyme-linked streptavidin linked to AP or HRP.

AGP-3R-related proteins or binding molecules may also be immobilized by attachment to agarose beads, acrylic beads or other types of such inert substrates. The substrate-protein complex can be placed in a solution containing the complementary protein and the test compound; after incubation, the beads can be precipitated by centrifugation, and the amount of binding between the AGP-3R-related protein and a binding molecule can be assessed using the methods described above. Alternatively, the substrate-protein complex can be immobilized in a

column and the test molecule and complementary molecule passed over the column. Formation of a complex between AGP-3R-related protein and the binding molecule can then be assessed using any of the techniques set forth above (i.e., radiolabeling, antibody binding, and the like).

Another useful in vitro assay is a surface plasmon resonance detector system, such as the Biacore assay system (Pharmacia, Piscataway, NJ). The Biacore system may be carried out using the manufacturer's protocol. This assay essentially involves covalent binding of either AGP-3 R related protein or a binding molecule to a dextran-coated sensor chip that is located in a detector. The test compound and the other complementary protein can then be injected into the chamber containing the sensor chip either simultaneously or sequentially and the amount of complementary protein that binds can be assessed based on the change in molecular mass that is physically associated with the dextran-coated side of the of the sensor chip; the change in molecular mass can be measured by the detector system.

In vitro assays such as those described above may be used advantageously to screen rapidly large numbers of compounds for effects on complex formation with AGP-3 R-related proteins. The assays may be automated to screen compounds generated in phage display, synthetic peptide and chemical synthesis libraries.

Compounds that increase or decrease complex formation among AGP-3R-related proteins and binding molecules may also be screened in cell culture using cells and cell lines bearing such ligands. Cells and cell lines may be obtained from any mammal, but preferably will be from human or other primate, canine, or rodent sources. Such cells may be enriched from other cell types by affinity chromatography using publicly available procedures. Attachment of AGP-3R-related protein to such cells is evaluated in the presence or absence of test compounds and the extent

of binding may be determined by, for example, flow cytometry using a biotinylated antibody. Cell culture assays may be used advantageously to further evaluate compounds that score positive in protein binding assays described above.

Description of Working Examples/Preferred Embodiments

The following examples are offered to illustrate the invention, but should not be construed as limiting the scope thereof.

Materials and Methods

Cloning of Human AGP-3

A TNF family profile search of the Genbank dbEST data base was performed. Smith et al.(1994), Cell, 76: 959-62; Luethy et al.(1994), Protein Science, 3: 139-46. One human EST sequence (GenBank accession number T87299) was identified as a possible new member of the TNF ligand. The EST was obtained from human fetal liver spleen cDNA library (The WashU-Merck EST Project). The cDNA clone (115371 3') corresponding to the EST sequence was obtained from Genome Systems, Inc. (St. Louis, MO). The cDNA fragment was released from the pT7T3D vector with EcoRI and NotI digestion. The fragment was approximately 0.7 kb in length and was used for the subsequent full-length cloning.

The ^{32}P-dCTP-labeled T87299 cDNA fragment was used as a probe to screen a human spleen cDNA phage library (Stratagene, La Jolla, CA). Recombinant phages were plated onto E. coli strain XL1-blue at approximately 5 x 10^4 transformants per 150 mm LB plate. Nitrocellulose filters were lifted from these plates in duplicates. Filters were prehybridized in 5x SSC, 50% deionized formamide, 5x Denhardt's solution, 0.5% SDS, and 100 µg/ml denatured salmon sperm DNA for 2 hours at 42 °C. The filters were then hybridized in the same solution with the addition of 5 ng/ml of labeled probe at 42°C overnight. The filters were first washed in 2x SSC and 0.1% SDS for 10 minutes at RT twice, and

then washed in 0.1x SSC and 0.1% SDS at 65 °C for 30 minutes twice. The filters were then exposed to autoradiography with intensifying screens at -80 °C overnight. Positive hybridizing plaques were determined by aligning the duplicate filters, and then picked up for subsequent secondary or tertiary screening till single isolated positive plaque was obtained. From total of one million recombinant phage clones, 8 positive plaques were obtained.

The pBluescript phagemid was excised from phage using the ExAssist™/SOLR™ System according to the manufacturer's description (Stratagene, La Jolla, CA). The excised phagemids were plated onto freshly grown SOLR cells on LB/ampicillin plates and incubated overnight. Single bacteria colony was amplified in LB media containing 100 μg/ml ampicillin. The plasmid DNA was prepared and both strands of cDNA insert were sequenced.

The human AGP-3 cDNA (clone 13-2) is 1.1 kb in length. It encodes a LORF of 285 amino acids. FASTA search of the SwissProt database with the predicted AGP-3 protein sequence indicated that it is mostly related to human TNFα with 25% identity in C-terminal 116 amino acid overlap. Like other TNF ligand family members, human AGP-3 protein is a type II transmembrane protein, containing a short N-terminal intracellular domain (amino acids 1-46), a hydrophobic transmembrane region (amino acids 47-68) following by a long C-terminal extracellular domain (amino acid 69-285). The C-terminal extracellular domain of AGP-3 contained most of the conserved region of the TNF ligand family. Smith et al.(1994), Cell, 76: 959-62.

Cloning of Murine AGP-3

An EST sequence (Genebank accession number AA254047) encoding a potential murine AGP-3 ortholog was identified by BLAST search of Genebank dbEST database with human AGP-3 sequence. The

corresponding cDNA clone (722549 5′) from mouse lymph node library was obtained from Genome Systems, Inc. (St. Louis, MO). The clone contained a 0.9 kb cDNA insert which could be released by EcoRI and NotI digestion. The 0.9 kb cDNA fragment encodes an open reading frame of 96 amino acids which shares 87% identity with the corresponding C-terminal human AGP-3 polypeptide sequence. A 0.41 kb EcoRI-XmnI fragment, which contained 290 bp coding region and 120 bp 3′ non-coding region, was used as probe to screening a mouse spleen cDNA phage library (Stratagene, La Jolla, CA) for full length murine AGP-3 cDNA as described above. From one million recombinant phage clones, 6 positive plaques were obtained. The phagemid was excised from phage as described above. The plasmid DNA was prepared and both strands of cDNA insert were sequenced. The murine AGP-3 cDNA (clone S6) encodes a polypeptide of 309 amino acids. Like its human ortholog, murine AGP-3 is also a type II transmembrane region, containing a short N-terminal intracellular domain (amino acid 1-46), a hydrophobic transmembrane region (amino acid 47-68) following by a long C-terminal extracellular domain (amino acid 69-285). The human and murine AGP-3 share 68% amino acid sequence identity overall. However, the C-terminal 142 amino acid sequences share 87% identity between the two species. Preceding the highly conserved C-terminus region, there is an insertion of 30 extra amino acids in the murine AGP-3. Four out of 7 positive phage plaques were independent clones, yet they all shared the same coding sequences.

Expression of human and murine AGP-3 mRNA

Multiple human or murine tissue northern blots (Clontech, Palo Alto, CA) were probed with ^{32}P-dCTP labeled human AGP-3 0.7kb EcoRI-NotI fragment or murine AGP-3 0.41kb EcoRI-XmnI fragment, respectively. The Northern blots were prehybridized in 5x SSC, 50%

deionized formamide, 5xDenhardt's solution, 0.5% SDS, and 100 μg/ml denatured salmon sperm DNA for 2 hours at 42 °C. The blots were then hybridized in the same solution with the addition of 5ng/ml of labeled probe at 42°C overnight. The filters were first washed in 2x SSC and 0.1% SDS for 10 minutes at RT twice, and then washed in 0.1x SSC and 0.1% SDS at 65 °C for 30 minutes twice. The blots were then exposed to autoradiography. The human tissue northern blot analysis with human AGP-3 probe under stringent conditions revealed predominant AGP-3 transcripts with a related molecular mass of 2.4kb in peripheral blood leukocytes (Figure 4A). Weaker expression was also detected in human spleen, lung and small intestine (Figure 4A). Among murine tissues analyzed, murine AGP-3 mRNA, with a relative molecular mass of 2kb, was mainly detected in spleen, lung, liver and kidney (Figure 4B).

Overexpression of murine AGP-3 in transgenic mice

Murine AGP-3 cDNA clone S6 in pBluescript SK(-) in pBluescript was used as template to PCR the entire coding region. T3 primer

5′ AAT TAA CCC TCA CTA AAG GG 3″

SEQ ID NO: 28

was used as 5′ PCR primer. The 3′ end PCR primer, which contained a XhoI site, was

5′ TCT CCC TCG AGA TCA CGC ACT CCA GCA AGT GAG 3′

SEQ ID NO: 29

PCR reactions were carried in a volume of 50 μl with 1 unit of vent DNA polymerase (New England Biolabs) in 20 mM Tris-HCl pH 8.8, 10 mM $(NH_4)_2SO_4$, 0.1% Triton-X100, 10 μM of each dNTP, 1 μM of each primer and 10 ng of murine AGP-3 cDNA template. Reactions were performed in 94 °C for 45 s, 55 °C for 55 S, and 72 °C for 2 minutes, for a total of 35 cycles. The PCR fragment created a XhoI site at 3′ end after the AGP-3 coding region. The 1 kb PCR fragment was purified by electrophoresis,

and digested with XbaI (present in the pBluescript MCS, 80 bp upstream of AGP-3 starting Methione) and XhoI restriction enzymes. The XbaI-XhoI PCR fragment was cloned into expression vector under the control of the human β-actin promoter. Graham et al.(1997), Nature Genetics 17: 272-4; Ray et al.(1991), Genes Dev. 5: 2265-73. The PCR fragment was sequenced to ensure no mutation. The murine AGP-3 expression plasmid was purified through two rounds of CsCl density gradient centrifugation. The purified plasmid was digested with ClaI, and a 6 kb fragment containing murine AGP-3 transgene was purified by gel electrophoresis. The purified fragment was resuspended in 5 mM Tris, pH 7.4, 0.2 mM EDTA at 2 µg/ml concentration. Single-cell embryos from BDF1 x BDF1-bred mice were injected as described (WO97 /23614). Embryos were cultured overnight in a CO_2 incubator and 15-20 2-cell embryos were transferred to the oviducts of pseudopregnant CD1 female mice.

Following term pregnancy, 62 offspring were obtained from implantation of microinjected embryos. The offspring were screened by PCR amplification of the integrated transgene in genomic DNA samples. Ear pieces were digested in 20 µl ear buffer (20mM Tris, pH8.0, 10mM EDTA, 0.5% SDS, 500 µg/ml proteinase K) at 55°C overnight. The sample was diluted with 200 µl of TE, and 2µl of the ear sample was used for the PCR reaction. The 5′ PCR primer

5′ AAC AGG CTA TTT CTT CAT CTA CAG 3′

SEQ ID NO: 30

resided in the murine AGP-3 coding region. The 3′ PCR primer

5′ CTC ATC AAT GTA TCT TAT CAT GTC T 3′

SEQ ID NO: 31

resided in the vector 3′ to the murine AGP-3 transgene. The PCR reactions were carried in a volume of 50 µl with 0.5 unit of Tag DNA polymerase (Boehringer Mannheim, Indianapolis, IN) in 10 mM Tris-HCl pH 8.3, 50

mM KCl, 2.5 mM $MgCl_2$, 10 μM of each dNTP, 1 μM of each primer and 2 μl of ear sample. The mixtures were first heated at 94 °C for 2 min, and the PCR reactions were performed in 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s, for a total of 35 cycles. Of the 62 offspring, 10 were identified as PCR positive transgenic founders.

At 8 weeks of age, all ten transgenic founders (animal 3, 6, 9, 10, 13, 38, 40, 58, 59, and 62) and five controls (animal 7, 8, 11, 12 and 14) were sacrificed for necropsy and pathological analysis. Portions of spleen were removed, and total cellular RNA was isolated from the spleens of all the transgenic founders and negative controls using the Total RNA Extraction Kit (Qiagen Inc., Chartsworth, CA). The expression of the transgene was determined by RT-PCR. The cDNA was synthesized using the SuperScript™ Preamplification System according to the manufacturer's instructions (Gibco BRL, Gaithersburg, MD). The primer

5′ CTC ATC AAT GTA TCT TAT CAT GTC T 3′

SEQ ID NO: 32

which was located in the expression vector sequence 3′ to the AGP-3 transgene, was used to prime cDNA synthesis from the transgene transcripts. Ten μg total spleen RNA from transgenic founders and controls were incubated with 1 μM of primer at 70°C for 10 min, and placed on ice. The reaction was then supplemented with 10 mM Tris-HCl pH 8.3, 50 mM KCL, 2.5 mM $MgCl_2$, 10 μM of each dNTP, 0.1 mM DTT and 200 U SuperScript II RT. After incubation at 42 °C for 50 min, the reaction was stopped by heating at 72 °C for 15 min. Total RNA were digested by addition of 2 U RNase H and incubation at 37 °C for 20 min. Subsequent PCR reactions were carried out by using murine AGP-3 specific primers. The 5′ PCR primer was

5′ AGC CGC GGC CAC AGG AAC AG 3′

SEQ ID NO: 33

The 3' PCR primer was

5' TGG ATG ACA TGA CCC ATA G 3'

SEQ ID NO: 34

The PCR reaction was performed in a volume of 50 µl with 0.5 unit Tag DNA polymerase in 10 mM Tris-HCl pH 8.3, 50mM KCL, 2.5mM $MgCl_2$, 10 µM of each dNTP, 1 µM of each primer and 1 µl of cDNA product. The reaction was performed at 94 °C for 30 s, 55°C for 30 S, and 72 °C for 1 min, for a total of 35 cycles. The PCR product was analyzed by electrophoresis. Transgene expression was detected in the spleen of all ten AGP-3 transgenic mice founders.

Biological activity of AGP-3

Prior to euthanasia, all animals were weighed, anesthetized by isofluorane and blood was drawn by cardiac puncture. The samples were subjected to hematology and serum chemistry analysis. The serum globulin level in all the AGP-3 transgenic mice (animal 3, 6, 9, 10, 13, 38, 40, 58, 59 and 62) increased more than 100% as compared to the control littermates (animal 7, 8, 11, 12 and 14, Table 1). Total protein level also increased correspondingly in the transgenic group, while albumin level remained the same. No significant differences in other serum chemistry or hematology parameters were observed at this age.

Radiography was performed after terminal exsanguination. There was no difference in the radiodensity or radiologic morphology of the skeleton. Upon gross dissection, major visceral organs were subject to weight analysis. The spleen weight relative to the body weight increased by approximately 45% in the AGP-3 transgenic group as compared to the control mice. The sizes of lymph nodes and Peyer's patches were also increased substantially in all the AGP-3 transgenic mice.

Following gross dissection, tissues were removed and fixed in 10% buffered Zn-Formalin for histological examination. The tissues collected

were liver, spleen, pancreas, stomach, the entire gastrointestinal tract, kidney, reproductive organs, skin and mammary glands, bone, brain, heart, lung, thymus, trachea, esophagus, thyroid, adrenals, urinary bladder, lymph nodes and skeletal muscle. After fixation, the tissues were processed into paraffin blocks, and 3 μm sections were obtained. All sections were stained with hematoxylin and exosin, and subject to histologic analysis. The size and the number of the follicles in the spleen, lymph nodes and Peyer's patches were increased significantly in the AGP-3 transgenic mice (Figure 5, 6 and 7). The spleen, lymph node and Peyer's patches of both the transgenic and the control mice were subject to immunohistology analysis with B cell and T cell specific antibodies. The formalin fixed paraffin embedded sections were deparaffinized and hydrated to deionized water. The sections were quenched with 3% hydrogen peroxide, blocked with Protein Block (Lipshaw, Pittsburgh, PA), and incubated in rat monoclonal anti-mouse B220 and CD3 (Harlan, Indianapolis, IN), respectively. The binding was detected by biotinylated rabbit anti-rat immunoglobulins and peroxidase conjugated streptavidin (BioGenex, San Ramon, CA) with DAB as chromagen (BioTek, Santa Barbara, CA). Sections were counterstained with hematoxylin. The B cell numbers, as indicated by positive B220 staining, increased significantly in the spleen, lymph nodes and Peyer's batches (Figure 5, 6, and 7). The T cell numbers, as indicated by the anti-CD3 staining, were slightly decreased. There were no differences in the morphology of the thymus between the transgenic and the control group. By immunohistology, the T cell population was similar in numbers. At 8 weeks of age, there are no distinctive morphologic changes in the liver, kidneys, or urinary, central nervous, hematopoietic, skeletal, respiratory, gastrointestinal, endocrine, or reproductive systems.

After necropsy, MLN and sections of spleen and thymus from 10 AGP-3 transgenic mice (animals 3, 6, 9, 10, 13, 38, 40, 58, 59 and 62) and 5 control littermates (animals 7, 8, 11, 12, and 14) were removed. Single cell suspensions were prepared by gently grinding the tissues with the flat end of a syringe against the bottom of a 100 μm nylon cell strainer (Becton Dickinson, Franklin Lakes, NJ). Cells were washed twice in a 15 ml volume then counted. Approximately 1 million cells from each tissue was stained with 0.5 μg antibody in a 100 μl volume of PBS (without Calcium and Magnesium) + 0.1% Bovine Albumin + 0.01% Sodium Azide. All spleen and MLN samples were incubated with 0.5 μg CD16/32(FcγIII/II) Fc block in a 20 μl volume for 10 minutes prior to the addition of FITC or PE-conjugated monoclonal antibodies against CD90.2 (Thy-1.2), CD45R (B220), CD11b(Mac-1), Gr-1, CD4, or CD8 (PharMingen, San Diego, CA) at 2-8 °C for 30 min. The cells were washed then analyzed by flow cytometry using a FACScan (Becton Dickinson, San Jose, CA). Thymus samples were stained with FITC conjugated anti-Thy-1.2, FITC conjugated anti-CD4, and PE conjugated anti-CD8 (PharMingen, San Diego, CA).

In the MLN of the AGP-3 transgenic mice, the percentage of B220 positive B cells increased by 100% (Figure 6). The percentage of the Thy-1.2 positive T cells decreased approximately 36%, with similar reductions in both CD4(+) and CD8(+) populations. The helper CD4(+) / suppressor CD8(+) ratio remained unchanged. Similar increases in B cell and reductions in T cell populations were also observed in the spleens of the AGP-3 transgenic mice (Figure 8), though to a lesser extent. No obvious changes in staining with anti-CD11b or anti-Gr-1 antibodies were observed in the lymph node and spleen between the transgenic and the control group. In the thymus, there were no differences in the percentages of Thy-1.2(+), CD4(+), CD8(+) or CD4(+)CD8(+) populations between the AGP-3 transgenic and the control mice.

Serum Immunoglobulin and Autoantibody Analysis

Transgenic mice and control littermates were bled successively at 6, 7, 8, 9, 11, and 12 weeks of age. Serum immunoglobulin levels were quantitated using by ELISA with Mouse Hybridoma Subtype Kit as suggested by manufacture (Boehringer Mannheim, Indianapolis, IN). Presence of autoantibodies directed against nuclear antigens and dsDNA were examined in the serum by enzyme linked immunosorbant assay (ELISA). The levels of anti-nuclear antibodies were detected using ANA screen kit (Sigma) and anti-mouse IgG peroxidase secondary antibody. Mouse serum samples were diluted 1:200 in ANA screen ELISA. For the detection of anti-dsDNA autoantibodies in serum, high binding ELISA plates were coated with plasmid DNA (Immunovision) as an antigen in the presence of methylated BSA. After blocking the non-specific sites and washing, diluted mouse serum samples were added to wells in duplicated and the binding was quantitated using horse radish peroxidase-labeled anti-mouse IgG or anti-mouse IgM reagents (Southern Biotech). A pooled positive serum from BWF1 mice and pooled negative serum from B6 mice was used as controls. Experiment for the detection of anti-histone antibodies was essentially done similar to anti-DNA ELISA except that carbonate-bicarbonate buffer (pH9.6) buffer was used as coating buffer. Serum antibody data were compared by Mann Whitney test using Sigmastat software (SPSS Science, Chicago, IL).

B Cell Survival and Proliferation Assay

Cells were isolated from spleens of 2-4 months old mice by negative selection. Briefly, B lymphocytes were purified by density gradient centrifugation and then passed over a B cells column (Accurate/ Cedarlane, Westbury, NY). Cells isolated by this method were analyzed by flow cytometry and >90% were found positive for B220 staining.

Isolated B cells were cultured in MEM+10% FCS at 37°C, 5%CO_2. Cells were collected from triplicate wells daily on day 1 through day 9 and incubated with 5 •g/ml Propidium Iodide. Cells were analyzed by Flow cytometry and the percentage of dead cells was calculated. For B cell proliferation assay, purified (10^5) B cells from B6 mice as described above were cultured in MEM+10% heat inactivated FCS in triplicate in 96 well flat bottomed plate with/without 2 μg/ml of Goat F(ab')$_2$ anti-mouse IgM (Jackson ImmunoResearch Laboratory, West Grove, Pennsylvania) and/or indicated amount of recombinant AGP-3 for a period of 4 days at 37 °C, 5%CO_2. Proliferation was measured by an uptake of radioactive ^{3}(H) thymidine in last 18 hours of pulse. Data is shown in figure 14 as mean±standard deviation of triplicate wells.

B Cell Hyperplasia and Hypergammaglobulinemia in AGP-3 Transgenic Mice

To gain insights into the biological function for AGP-3, transgenic mice were generated that expressed full-length murine AGP-3 protein driven by the ubiquitous 3-actin promoter. Founder mice harboring the AGP-3 transgene were identified by PCR analysis of genomic DNA samples. Transgene expression was confirmed by RT-PCR from spleen total RNA. At 8 weeks of age, ten AGP-3 transgenic mice and five control littermates were subject to necropsy and pathological analysis. The transgenic mice were of normal size and weight. However, the spleen weight relative to the body weight increased by approximately 45% in the AGP-3 transgenic group as compared to the control mice. The sizes of lymph nodes and Payer's Patches were also increased substantially in all the AGP-3 transgenic mice. Histology analysis demonstrated that the size and the number of the follicles in the spleen, lymph nodes and Payer's patches were increased significantly in the AGP-3 transgenic group

(Figure 10). Immunohistology staining with B and T cell specific markers indicated the B cell numbers increased significantly in the spleen, lymph nodes and Payer's patches of the transgenic group (Figure 10). The T cell numbers, as indicated by the anti-CD3 staining, were decreased correspondingly (Figure 10). There were no differences in the morphology and immunostaining of thymus between the transgenic and the control groups. No changes were observed in other organs or organ systems of the 8 weeks old transgenic mice, including kidney, liver, and hematopoietic tissues.

The B cell hyperplasia phenotype in the AGP-3 transgenic mice was also confirmed by flow cytometry analysis. In the mesenteric lymph nodes of the AGP-3 transgenic mice, the percentage of B220 positive B cells increased by 100% (Figure 11). The percentage of the Thy-1.2 positive T cells decreased by approximately 36%, with similar reductions in both CD4(+) and CD8(+) T cells. Similar increase in B cell and reduction in T cell populations were also observed in the spleens of the AGP-3 transgenic mice, though to a lesser extent (Figure 11). Of note, the total T cell numbers in the lymph node and spleen of AGP-3 transgenic mice were similar to the control littermates. In the thymus, there were no differences in the percentages of single positive CD4(+) or CD8(+) T cells, or CD4(+)CD8(+) populations between the AGP-3 transgenic and the control mice (Figure 11). No obvious changes in staining with anti-CD11b or anti-Gr-1 antibodies were observed in the lymph nodes and spleen between the transgenic and the control group (Figure 11). The histological and FACS analysis, together, suggested severe B cell hyperplasia phenotype in the AGP-3 transgenic mice.

We also examined B cell populations of different developmental stages by FACS analysis. No differences were observed in the percentage of the pro B (B220+IgM-), immature B (B220+IgM+), or mature B

(IgM+IgD+) within spleenic B cell population of the AGP-3 transgenic mice as compared to the control littermates. In addition, the number of the spleenic CD5+ B cells in the AGP-3 transgenic mice from 1 to 9 month of age was unaltered. We also didn't detect any alteration of the CD40 expression level on B cells in the transgenic mice, suggesting that the B cell hyperplasia in the AGP-3 transgenic mice was not caused by CD40 upregulation.

In addition to the B cell hyperplasia phenotype, the AGP-3 transgenic mice also had severe hypergammaglobulinemia . The serum globulin level in AGP-3 transgenic mice increased more that 100% as compared to the control group. Total protein level also increased correspondingly in the transgenic, while albumin level remained the same. The increased B cell numbers and high serum globulin level suggested elevated serum immunoglobulin titer. Thus we examined serum levels of IgM, IgG, IgA and IgE of AGP-3 transgenic mice from 6 to 12 weeks of age. Comparing to the same age control littermates, serum IgM, IgG, IgA and IgE were significantly increased in all age groups of AGP-3 transgenic mice. The increase found in serum IgG was not specific to any particular subclass (IgG1, IgG2a, IgG2b, and IgG3). No significant differences in other serum chemistry or hematology parameters were observed at this age. The increased serum immunoglobulin levels is likely to result directly from increased B cell number, but may also be aggravated by increased B cell antibody production.

Autoantibodies associated with lupus in AGP-3 transgenic mice

Increased humoral immunity in AGP-3 transgenic mice warranted us to look for possible phenotypes resembling B cell associated autoimmune diseases such as systemic lupus erythematosus (SLE). The common denominator in lupus patients and lupus prone mice is IgG autoantibody production, and the hallmark of this disease is the presence

of elevated anti-nuclear antibodies in the serum. The emergence of anti-DNA antibodies represents one final outcome in the different murine lupus models and patients with SLE. When sera from transgenic and non-transgenic mice at various age were examined for the presence of autoantibodies recognizing nuclear antigens or dsDNA, two different lines of AGP-3 transgenic mice began to show presence of autoantibodies at around 8 weeks of age (Table 1). The amount of anti-nuclear and anti-dsDNA antibody increased with their age in the transgenic animals (Table 1). More interestingly, at 5 and 8 months of age, AGP-3 transgenic mice showed 5-10 higher amount of anti-dsDNA antibodies compared to age matched lupus prone (NZBxNZW)F1 mice. The presence of autoantibodies in the serum of AGP-3 transgenic mice did not discriminate between gender of mice. Both IgG and IgM antibodies to dsDNA were detected in transgenic animals. Presence of such autoantibodies was undetectable in non-transgenic littermates, as expected.

Immune Complex Deposits in the Kidney of AGP-3 Transgenic Mice

Presence of anti-DNA antibodies followed by immune complex induced renal damage is classical picture seen in lupus associated nephritis. At 5 month of age, the AGP-3 transgenic mice developed glomerular proteinaceous deposits in the kidney (Figure 13). The deposits were seen in more than 60% of the glomeruli in the transgenic mice, but absent in the control littermates. Immunohistology showed the deposits contained moderate amounts of IgG and larger amounts of IgM (Figure 13). Trichrome staining showed no deposit of connective tissues in the glomeruli at 5 month of age. There is also no evidence of any cellular proliferation or presence of inflammatory cells at this age (Figure 13). Interestingly, the kidney lesions progressed as the transgenic mice grew

older. At 8 month of age, there was obvious enlargement of glomeruli in the AGP-3 transgenic mice as compared to the age matched control littermates (Figure 13G). In addition, we also detected extensive connective tissue deposits in the enlarged glomeruli (Figure 13G). Comparing to the 5 month old mice, the 8 month old transgenic mice had increased IgG level in the glomeruli immune complex deposits (Figure 13I). Majority of the glomeruli in the AGP-3 transgenic mice were affected. We also performed serum and urine chemistry analysis of 5 month old and 8 month old AGP-3 transgenic along with the control littermates. No significant differences were noticed in the 5 month old AGP-3 transgenic mice. However, in the 8 month old mice, we observed increases in serum blood urea nitrogen (BUN) and calcium levels and decrease in serum phosphate level. In addition, the 8 month old AGP-3 mice also had increased protein level in the urine. These changes, together, suggest the onset of renal failure in the 8 month old AGP-3 transgenic mice. In conclusion, the high serum autoantibodies followed by the kidney lesions in theAGP-3 transgenic mice clearly resemble to the pathological progression in the SLE patients and lupus prone mice.

AGP-3 Stimulates B Cell Survival and Proliferation : a Possible Mechanism for Autoimmunity

The B cell hyperplasia phenotype in the AGP-3 transgenic mice might arise from increased B cell survival and/or increased B cell proliferation. We first compared the viability of B cells from AGP-3 transgenic mice with that of the control littermates. B cells were isolated from both transgenic or control mice and incubated in minimal essential medium supplemented with 10% heat inactivated fetal bovine serum. Viability of the B cells was measured by FACS analysis for Propidium Iodide uptake (Figure 14A). By day 3, 30% of B cells isolated from the control mice were dead, whereas only 10% of B cells from AGP-3

transgenic mice were dead. By day 5, 70% of B cells from AGP-3 mice were still viable, whereas only 15% of B cell from control littermates were viable. By day 9, almost 50% of the AGP-3 transgenic B cells still remained viable. Therefore, transgenic expression of AGP-3 prolonged B cell viability. It remains to be determined if this B cell survival stimuli result directly from AGP-3 action on B cells or through its modulation of the immune system.

Recently Schneider et al (Schneider et al., 1999, and Moore et al., 1999) reported co-stimulation of B cell proliferation by BAFF/BLYS with anti-IgM. We found that AGP-3 alone can also stimulates B cell proliferation in a dose dependent manner with an ED_{50} of approximately 3ng/ml (Figure 14B, upper). A ten fold increase of B cell proliferation was detected by AGP-3 treatment at 10 ng/ml concentration as compared to the untreated cells. In our experiment, anti-IgM alone at 2 μg/ml concentration increased B cell proliferation by 24 fold. Treatment with anti-IgM (2 μg/ml) in combination with various doses of AGP-3 led to dose dependent increase of B cell proliferation, with a maximal 13 fold increase as compared anti-IgM treatment alone and a total of 320 fold increase as compared to the untreated cells. Thus, AGP-3 is a potent B cell stimulatory factor. The increased B cell survival and proliferation may together contribute to the B cell hyperplasia and autoimmune lupus like changes in the AGP-3 transgenic mice.

Table 1: Lupus associated autoantibodies in the serum of AGP-3 transgenic mice.

Autoantibodies	Age (months)	AGP-3 tg (n)	Non-tg littermates (n)	p value
Antinuclear antibodies (IgG)[a]	2-3	7^ (9)	1*(8)	
	5-6	9 (9)	1*(8)	
	8-9	8 (8)	1*(6)	
Anti-dsDNA (IgG)[b]	<2	697±284 (7)	277±67 (7)	NS
	3-4	842±351 (7)	235±49 (7)	<.005
	6-7	2515±428 (5)	970±344 (7)	<.019
	8-10	12293±6767 (11)	1070±602 (12)	<.017
Anti-dsDNA (IgM)[b]	<2	275±33 (7)	46±5 (7)	<.001
	3-4	1684±920 (7)	63±13 (7)	<.003
	6-7	6998±5515 (5)	98±14 (7)	<.001
	8-10	13712±9147 (11)	79±14 (12)	<.001
Anti-Histone (Ig)[b]	<2	741±264 (7)	52±8 (7)	<.001
	3-4	837±436 (7)	53±14 (7)	<.003
	6-7	4220±933 (5)	60±10 (7)	<.001
	8-10	16555±4618 (11)	295±173 (12)	<.001

^ includes two weak positive.

* Weak positive

a: Data is shown as number of ANA positive (mean+2sd of transgene negative littermates) mice using ANA screen kit.

b: Data is represented as mean±SE for each group. Values are shown as Units/ml.

NS: not significant

Bacterial Expression of AGP-3 protein

PCR amplification employing the primer pairs and templates described below are used to generate various forms of human AGP-3 proteins. One primer of each pair introduces a TAA stop codon and a unique XhoI or SacII site following the carboxy terminus of the gene. The other primer of each pair introduces a unique NdeI site, a N-terminal methionine, and optimized codons for the amino terminal portion of the gene. PCR and thermocycling is performed using standard recombinant DNA methodology. The PCR products are purified, restriction digested, and inserted into the unique NdeI and XhoI or SacII sites of vector pAMG21 (ATCC accession no. 98113) and transformed into the prototrophic E. coli 393 or 2596. Other commonly used E. coli expression vectors and host cells are also suitable for expression. After transformation, the clones are selected, plasmid DNA is isolated and the sequence of the AGP-3 binding protein insert is confirmed.

pAMG21-Human AGP-3 protein [128-285]

This construct was engineered to be C-terminal 158 amino acids of human AGP-3 and have the following N-terminal and C-terminal residues:

NH_2-MNSRNKR ——GALKLL-COOH.

(SEQ ID NOS: 35 and 52, respectively)

The template to be used for PCR was human AGP-3 in pCDNA3.1(+) vector. Oligonucleotides #1761-31 and #1761-33 were the primer pair to be used for PCR and cloning this gene construct.

1761-31:

5'-ATT TGA TTC TAG AAG GAG GAA TAA CAT ATG AAC AGC CGT AAT AAG CGT GCC GTT CAG GGT -3'

(SEQ ID NO:_36)

1761-33:

5'-CCG CGG ATC CTC GAG TTA CAG CAG TTT CAA TGC ACC AAA AAA TGT -3'

(SEQ ID NO:_37)

pAMG21-Human FLAG-AGP-3 protein [128-285]

This construct was engineered to be C-terminal 158 amino acids of human AGP-3 preceded with FLAG epitope. The construct encoded following following N-terminal and C-terminal residues:

NH_2-MDYKDDDDKKLNSRNKR-------GALKLL-COOH

(SEQ ID NOS: 38 and 52)

The template to be used for PCR was human AGP-3 in pCDNA3.1(+) vector. Oligonucleotides #1761-32 and #1761-33 were the primer pair to be used for PCR and cloning this gene construct.

1761-32:
5'-GAC GAT GAC AAG AAG CTT AAC AGC CGT AAT AAG CGT GCC GTT CAG GGT -3'

(SEQ ID NO:39)

1761-33:
5'-CCG CGG ATC CTC GAG TTA CAG CAG TTT CAA TGC ACC AAA AAA TGT -3'

(SEQ ID NO:_38)

E. coli were induced during fermentation, the lysate was applied to Q Sepharose FF (Pharmacia, Piscaataway, NJ) equilibrated in 10 mM Mes pH 6.0 and eluted with 50- 400 mM NaCl gradient over 30 column volumes. Fractions containing AGP-3 were pooled and loaded onto a Q Sepharose HP column (Pharmacia, Piscataway, NJ) equilibrated in 10 mM Tris-HCL pH 8.5. AGP-3 was eluted with an increasing linear NaCl gradient (50 mM-200 mM) over 30 column volumes. Endotoxin was removed by application to Sp HiTRAP column (Pharmacia, Piscataway, NJ) pH 4.8 and eluted with 100-500 mM NaCl in 10 mM sodium acetate

pH 4.8 over 25 column volumes. Final endotoxin level of the purified protein is approximately 0.2 EU/mg. The purified human AGP-3 is truncated at residue Arg133 as indicated by N-terminal sequencing and has a molecular weight of 16.5 KDa by reducing SDS-PAGE. The purified human FLAG-AGP-3 protein is confirmed by N-terminal sequence analysis of the protein. The FLAG-AGP-3 protein is recognized by M2 monoclonal antibody against FLAG epitope (Kodak, New Haven, CT).

For europium labeling of the protein, human AGP-3 (lot# 092299) was dialyzed into 50 mM sodium carbonate pH 9. Europium labeling reagent (Wallac Delfia reagent lot# 704394) was dissolved in the same buffer. AGP-3 protein was mixed with a 20-fold molar excess of labeling reagent for 24 hours at room temperature. The mixture was then placed on a Sephadex G-25 column which had been equilibrated in 50 mM Tris-HCl pH 7.8, 150 mM NaCl. The protein was eluted from the column with the same buffer. Protein concentration was determined using the BCA method (Pierce Chemical Co.).

Expression cloning of AGP-3 receptor

AGP-3 induces B cell proliferation and survival, suggesting the presence of its receptor on primary B cells or B cell lines. To identify a source of AGP-3 receptor, recombinant FLAG-AGP-3 protein was used as immunoprobe to screen for its receptor located on the surface of various cell lines and primary hematopoietic cells. Cells were harvested from exponentially replicating cultures in growth media, pelleted by centrifugation, washed with phosphate buffered saline (PBS) (Gibco) containing 1% fetal calf serum (FCS), and then resuspended at 1 X 10^7 cells/ml in a 96 well microtiter tissue culture plate (Falcon) in PBS with 1% FCS containing 1 μg/ml FLAG-AGP-3. After 1 hour incubation at 4°C, cells were washed with PBS with 1%FCS, and then incubated in PBS with 1%FCS containing 20 μg/ml anti-FLAG M2 monoclonal antibody (Kodak,

New Haven, CT) for 30 minutes at 4°C. After washing with PBS, cells were incubated again in PBS with 1% FCS containing 20 μg/ml FITC-conjugated goat anti-mouse IgG (Southern Biotech Associates, Birmingham, AL) for 30 minutes at 4°C. After washing, cells were then analyzed by fluorescence activated cell sorting (FACS) using a Becton Dickinson FACscan. The specificity of the binding was confirmed by addition of 100 μg/ml AGP-3 protein during the first incubation period.

Using this approach, human Burkitt lymphoma RAJI and BJAB cells, and human lymphoblast GM3104A were found to express a surface molecule which could be detected by FLAG-AGP-3 (Figure 15). Secondary antibody alone did not bind to the surface of these cell lines. This binding could be competed in a dose dependent manner by the addition of non-FLAG tagged AGP-3 protein. The binding by FLAG-AGP-3 protein was not detected on human acute T lymphoblastic leukemic Molt3 cells.

A cDNA library was prepared from 32D mRNA, and ligated into a mammalian expression vector. Exponentially growing RAJI were harvested, and total cell RNA was purified by acid guanidinium thiocyanate-phenol-chloroform extraction (Chomczynski and Sacchi. Anal. Biochem. 162, 156-159, (1987)). The poly (A+) mRNA fraction was obtained from the total RNA preparation by adsorption to, and elution from, Dynabeads Oligo (dT)25 (Dynal Corp) using the manufacturer's recommended procedures. A directional, oligo-dT primed cDNA library was prepared using the Superscript Plasmid System (Gibco BRL, Gaithersburg, Md) using the manufacturer's recommended procedures. The resulting cDNA was digested to completion with Sal I and Not I restriction endonuclease, then fractionated by size exclusion gel chromatography. The highest molecular weight fractions were selected, and then ligated into the polylinker region of the expression vector. This

vector contains the CMV promoter upstream of multiple cloning site, and directs high level expression in eukaryotic cells. The library was then electroporated into competent E. coli (ElectroMAX DH10B, Gibco, NY), and titered on LB agar containing 100 μg/ml ampicillin. The library was then arrayed into segregated pools containing approximately 100 clones/pool, and 1.0 ml cultures of each pool were grown for 16-20 hr at 37°C. Plasmid DNA from each culture was prepared using the Qiagen Qiawell 96 Ultra Plasmid Kit (catalog #16191) following manufacturer's recommended procedures.

Arrayed pools of RAJI cDNA expression library were individually transfected into 293 cells (ATCC), then assayed for the acquisition of a cell surface AGP-3 binding protein using MultiPitte™ (Sagian Inc.). To do this, 293 cells were plated at a density of 1.5 X 10^4 per ml in 96-well tissue culture plates (Falcon), then cultured overnight in DMEM (Gibco) containing 10% FCS. Approximately 300ng of plasmid DNA from each pool was diluted into 75 μl of OPTI-MEMI Reduced Serum Medium (Life Technologies, Gaithersburg, MD). Simultaneously, 1 μl of DMRIE-C (Life Technologies, Gaithersburg, MD) was added to diluted into 75 μl of OPTI-MEMI Reduced Serum Medium. The DNA and DMRIE-C solutions were mixed, and allowed to incubate at room temperature for 30 min. The 293 cell cultures were exposed to the DNA-DMRIE-C complexes for 2-5 hr at 37°C. After this period, the cells supplemented with equal volume of DMEM containing 20%FCS. The cells were then cultured for 48 hr at 37°C.

To detect cultures that express an AGP-3 binding protein, the growth media of each well was removed and replaced with 100 μl DMEM containing 2% goat serum, 5% rabbit serum (Life Technologies, Gaithersburg, MD) and 0.1nM europium-labeled AGP-3 protein. Cells were incubated at RT for 1 hr. The cells were washed three times with 175

µl cold PBS, and then resuspended with 170 µl of Enhancer Solution (EG&G Wallac, Turku, Finland). The 96 well plates were then subject to analysis by Victor™ 1420 Multiplabel Counter (Wallac, Inc., Gaithersburg, MD).

Using this approach, a total of approximately 300,000 independent RAJI cDNA clones were screened, represented by 3000 transfected pools of 100 clones each. Transfection and binding assay of each pool was performed in duplicates. Six wells were identified that contained cells which acquired the ability to be specifically decorated by the Europium-labeled AGP-3 protein. The positive signals ranged from 2-10 fold (Table 2). Five hundred bacteria colonies were picked for positive pool 13B4 and pool 13H11. The bacteria were cultured overnight. Plasmid DNA from each culture was prepared using the Qiagen Qiawell 96 Ultra Plasmid Kit (catalog #16191) following manufacturer's recommended procedures. Each plasmid prepreparation was transfected to 293 cells and examined for binding activity with europium-labeled AGP-3 as described above. Sixteen out of 500 clones of pool 13B4 were positive for AGP-3 binding, and 8 out of 500 clones of pool 13H11 were positive. Positive binding clones of both 13B11 and 13H4 pools were subject to sequence analysis. The positive binding clones from 13B11 and 13H4 pools encoded same gene, the latter has extra 7 basepairs at the N-terminus (Figure 16).

AGP-3 receptor DNA and protein sequence

The RAJI-13H4 clone isolated above contained an approximately 1.6 kb cDNA insert (Figure 15), which was sequenced in both directions on an Applied Biosystems 373A automated DNA sequencer using primer-driven Taq dye-terminator reactions (Applied Biosystems) following the manufacturer's recommended procedures. The resulting nucleotide sequence obtained was compared to the DNA sequence database using the FASTA program (GCG, Univeristy of Wisconsin), and analyzed for the

presence of long open reading frames (LORF's) using the "Six-way open reading frame" application (Frames) (GCG, Univeristy of Wisconsin). A LORF of 293 amino acid (aa) residues beginning at methionine was detected in the appropriate orientation, and was preceded by a 5' untranslated region of about 17 bp and an in-frame stop codon upstream of the predicted start codon (Figure 17). The RAJI-13B4 clone encodes a LORF of the same 293 amino acid residues, with 5' 10 bp untranslated region and an in-frame stop codon. This indicates that the structure of the RAJI- plasmid is consistent with its ability to utilize the CMV promoter region to direct expression of a 293 aa gene product in mammalian cells.

The AGP-3 receptor contains a probably hydrophobic transmembrane domain that begins at a T166 and extends to L186. Based on this configuration relative to the methionine start codon, the AGP-3 receptor is predicted to be a type III transmembrane protein, with a N-terminal extracellular domain, a transmembrane region and a C-terminal intracellular domain. Unlike most other TNFR receptor family members, AGP-3 receptor contains two cysteine rich repeats within its N-terminal extracellular domain (Figure 4).

The predicted AGP-3 receptor protein sequence was then compared to the existing database of known protein sequences using a modified version of the FASTA program (Pearson, Meth. Enzymol. 183, 63-98 (1990)). The amino acid sequence was also analyzed for the presence of specific motifs conserved in all known members of the tumor necrosis factor receptor (TNFR) superfamily using the sequence profile method of (Gribskov et al. (1987), Proc. Natl. Acad. Sci. USA 83, 4355-9), as modified by Lüethy et al.(1994), Protein Sci. 3, 139-146 .

Expression of human AGP-3 receptor mRNA

Multiple human tissue northern blots (Clontech, Palo Alto, CA) were probed with a ^{32}P-dCTP labeled AGP-3 receptor restriction fragment

to detect the size of the human transcript and to determine patterns of expression. Northern blots were prehybridized in 5X SSPE, 50% formamide, 5X Denhardt's solution, 0.5% SDS, and 100 μg/ml denatured salmon sperm DNA for 2-4 hr at 42°C. The blots were then hybridized in 5X SSPE, 50% formamide, 2X Denhardt's solution, 0.1% SDS, 100 μg/ml denatured salmon sperm DNA, and 5 ng/ml labeled probe for 18-24 hr at 42°C. The blots were then washed in 2X SSC for 10 min at RT, 1X SSC for 10 min at 50°C, then in 0.5X SSC for 10-15 minutes.

Using a probe derived from a 1.5kb SalI NotI fragment of human AGP-3 receptor and hybridization under stringent conditions, a predominant mRNA species with a relative molecular mass of about 2.4 kb was detected in spleen, lymph nodes, skeletal muscle and heart (Figure 20).

Production of Recombinant AGP-3 receptor Protein in Mammalian Cells

Expression construct was generated that direct synthesis of AGP-3 receptor extracellular domain fused with the Fc region of human. The following sets of oligonucleotide primers were used to PCR extracellular domain of human AGP-3 receptor (amino acids 1-166) was PCR amplified with the following set of oligonucleotide primers:

5' TCT CCA AGC TTC CGA TCC TGA GTA ATG AGT GG -3'
(SEQ ID NO: 50)
5' TCT CCG CGG CCG CGC TGT AGA CCA GGG CCA CCT G-3'
(SEQ ID NO: 51)

PCR reactions were carried in a volume of 50 μl with 1 unit of vent DNA polymerase (New England Biolabs) in 20 mM Tris-HCl pH 8.8, 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 0.1% Triton-X100, 10 μM of each dNTP, 1 μM of each primer and 10 ng of ODAR cDNA template. Reactions were

performed in 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min, for a total of 16 cycles. The PCR fragment was isolated by electrophoresis. The PCR fragment creates a Hind III restriction site at 5′ end and a Not I restriction site at 3′ end. The Hind III-Not I digested PCR fragment was then subcloned in-frame into a modified Fc/pCEP4 vector in front of the human IgG-γ1 heavy chain sequence as described previously in WO97/23614 and in Simonet et al. supra).

The construct was transfected into 293-EBNA-1 cells by calcium phosphate method as described (Ausubel et al. (1994), Curr. Prot. Mol. Biol. 1, 9.1.1-9.1.3. Both medium and cell lysates from transfected 293 cells were subject to western analysis with rabbit anti-human IgG Fc polyclonal antibody and subsequently with horseradish peroxidase linked anti-rabbit antibody (Amersham, Piscataway, NJ). The extracellular domain of AGP-3 receptor fused with Fc domain was detected only in the cell lysates could be immunoprecipitated with AGP-3 protein. This finding supports that AGP-3 receptor is a type III transmembrane domain, which doesn't have an N-terminal signal peptide (Figure 21). The receptor is directed to cell surface probably by an internal signal anchor sequence. Soluble receptor protein could be generated by grafting with an N-terminal signal peptide.

Abbreviations

Abbreviations as used throughout this specification are defined as follows, unless otherwise defined in specific instances.

CDR	complementarity determining region
dsDNA	double-stranded DNA
EST	expressed sequence tag
FCS	fetal calf serum
ORF	open reading frame
PBS	phosphate-buffered saline
SDS	sodium dodecyl sulfate

TNF tumor necrosis factor

* * *

While the present invention has been described in terms of the preferred embodiments, it is understood that variations and modifications will occur to those skilled in the art. Therefore, it is intended that the appended claims cover all such equivalent variations that come within the scope of the invention as claimed.

What is claimed is:

1. A composition of matter comprising the structure

$$(X^1)_a\text{-}F^1\text{-}(X^2)_b$$

wherein:

F^1 is a vehicle;

X^1 and X^2 are each independently selected from $-(L^1)_c\text{-}P^1\text{-}(L^2)_d\text{-}P^2$, $-(L^1)_c\text{-}P^1\text{-}(L^2)_d\text{-}P^2\text{-}(L^3)_e\text{-}P^3$, and $-(L^1)_c\text{-}P^1\text{-}(L^2)_d\text{-}P^2\text{-}(L^3)_e\text{-}P^3\text{-}(L^4)_f\text{-}P^4$

P^1, P^2, P^3, and P^4 are each independently selected from SEQ ID NOS: 45 and 46;

L^1, L^2, L^3, and L^4 are each independently linkers; and

a and b are each independently 0 or 1, provided that at least one of a and b is 1;

c, d, e, and f are each independently 0 or 1, provided that if P^1 is SEQ ID NO: 45 and P^2 is SEQ ID NO: 46, then d is 1;

and wherein said composition of matter does not comprise SEQ ID NO: 43.

2. The composition of matter of Claim 1 of the formulae

$$X^1\text{-}F^1$$

or

$$F^1\text{-}X^2.$$

3. The composition of matter of Claim 1 of the formula

$$F^1\text{-}(L^1)_c\text{-}P^1\text{-}(L^2)_d\text{-}P^2.$$

4. The composition of matter of Claim 1 wherein F^1 is an Fc-region.
5. The composition of matter of Claim 1 wherein F^1 is an IgG Fc domain.
6. The composition of matter of Claim 1 wherein F^1 is an IgG1 Fc domain.
7. The polypeptide of Claim 1, wherein F^1 is a water-soluble polymer or a carbohydrate.
8. The protein of Claim 7, wherein the polymer is polyethylene glycol.

9. The protein of Claim 7, wherein the carbohydrate is dextran.
10. A polypeptide of Claim 1 capable of eliciting B cell growth, survival, or activation in mesenteric lymph nodes.
11. An isolated nucleic acid encoding a polypeptide of Claim 1.
12. The nucleic acid of Claim 11 including one or more codons preferred for Escherichia coli expression.
13. The nucleic acid of Claim 11 having a detectable label attached thereto.
14. An expression vector comprising the nucleic acid of Claim 11.
15. A host cell comprising the expression vector of Claim 14.
16. The host cell of Claim 15, wherein the cell is a prokaryotic cell.
17. The host cell of Claim 16, wherein the cell is Escherichia coli.
18. A pharmaceutical composition comprising a therapeutically effective amount of a protein of Claim 1 in a pharmaceutically acceptable carrier, adjuvant, solubilizer, stabilizer and/or anti-oxidant.
19. A method of modulating AGP-3-related activity in a mammal, which comprises administering a therapeutically effective amount of the composition of matter of Claim 1.
20. The method of Claim 22, wherein the AGP-3-related activity takes place in mesenteric lymph nodes.
21. A polypeptide comprising an antibody sequence in which one or more amino acids from antibody variable domains or CDR regions are replaced by sequences selected from SEQ ID NOS: 45 and 46.
22. The polypeptide of Claim 21, wherein a first CDR region is replaced by SEQ ID NO: 45 and a second CDR region is replaced by SEQ ID NO: 46.
23. The polypeptide of Claim 21, wherein all CDR regions are replaced by SEQ ID NO: 45.
24. An isolated nucleic acid encoding a polypeptide of Claim 21.
25. The nucleic acid of Claim 24 having a detectable label attached thereto.

26. An expression vector comprising the nucleic acid of Claim 24.

27. A host cell comprising the expression vector of Claim 26.

28. A pharmaceutical composition comprising a therapeutically effective amount of a polypeptide of Claim 21 in a pharmaceutically acceptable carrier, adjuvant, solubilizer, stabilizer and/or anti-oxidant.

29. A method of modulating AGP-3-related activity in a mammal, which comprises administering a therapeutically effective amount of the composition of matter of Claim 21.

30. The method of Claim 29, wherein the AGP-3-related activity takes place in mesenteric lymph nodes.

FIG. 1A

```
        10                  30                  50
GAATTCGGCACGAGCTGAGGGGTGAGCCAAGCCCTGCCATGTAGTGCACGCAGGACATCA
        70                  90                  110
ACAAACACAGATAACAGGAAATGATCCATTCCCTGTGGTCACTTATTCTAAAGGCCCCAA
       130                 150                 170
CCTTCAAAGTTCAAGTAGTGATATGGATGACTCCACAGAAAGGGAGCAGTCACGCCTTAC
                        M   D   D   S   T   E   R   E   Q   S   R   L   T
       190                 210                 230
TTCTTGCCTTAAGAAAAGAGAAGAAATGAAACTGAAGGAGTGTGTTTCCATCCTCCCACG
 S   C   L   K   K   R   E   E   M   K   L   K   E   C   V   S   I   L   P   R
       250                 270                 290
GAAGGAAAGCCCCTCTGTCCGATCCTCCAAAGACGGAAAGCTGCTGGCTGCAACCTTGCT
 K   E   S   P   S   V   R   S   S   K   D   G   K   L   L   A   A   T   L   L
       310                 330                 350
GCTGGCACTGCTGTCTTGCTGCCTCACGGTGGTGTCTTTCTACCAGGTGGCCGCCCTGCA
 L   A   L   L   S   C   C   L   T   V   V   S   F   Y   Q   V   A   A   L   Q
       370                 390                 410
AGGGGACCTGGCCAGCCTCCGGGCAGAGCTGCAGGGCCACCACGCGGAGAAGCTGCCAGC
 G   D   L   A   S   L   R   A   E   L   Q   G   H   H   A   E   K   L   P   A
       430                 450                 470
AGGAGCAGGAGCCCCCAAGGCCGGCCTGGAGGAAGCTCCAGCTGTCACCGCGGGACTGAA
 G   A   G   A   P   K   A   G   L   E   E   A   P   A   V   T   A   G   L   K
       490                 510                 530
AATCTTTGAACCACCAGCTCCAGGAGAAGGCAACTCCAGTCAGAACAGCAGAAATAAGCG
 I   F   E   P   P   A   P   G   E   G   N   S   S   Q   N   S   R   N   K   R
       550                 570                 590
TGCCGTTCAGGGTCCAGAAGAAACAGTCACTCAAGACTGCTTGCAACTGATTGCAGACAG
 A   V   Q   G   P   E   E   T   V   T   Q   D   C   L   Q   L   I   A   D   S
```

FIG. 1B

```
      610                 630                 650
TGAAACACCAACTATACAAAAAGGATCTTACACATTTGTTCCATGGCTTCTCAGCTTTAA
 E  T  P  T  I  Q  K  G  S  Y  T  F  V  P  W  L  L  S  F  K
      670                 690                 710
AAGGGGAAGTGCCCTAGAAGAAAAAGAGAATAAAATATTGGTCAAAGAAACTGGTTACTT
 R  G  S  A  L  E  E  K  E  N  K  I  L  V  K  E  T  G  Y  F
      730                 750                 770
TTTTATATATGGTCAGGTTTTATATACTGATAAGACCTACGCCATGGGACATCTAATTCA
 F  I  Y  G  Q  V  L  Y  T  D  K  T  Y  A  M  G  H  L  I  Q
      790                 810                 830
GAGGAAGAAGGTCCATGTCTTTGGGGATGAATTGAGTCTGGTGACTTTGTTTCGATGTAT
 R  K  K  V  H  V  F  G  D  E  L  S  L  V  T  L  F  R  C  I
      850                 870                 890
TCAAAATATGCCTGAAACACTACCCAATAATTCCTGCTATTCAGCTGGCATTGCAAAACT
 Q  N  M  P  E  T  L  P  N  N  S  C  Y  S  A  G  I  A  K  L
      910                 930                 950
GGAAGAAGGAGATGAACTCCAACTTGCAATACCAAGAGAAAATGCACAAATATCACTGGA
 E  E  G  D  E  L  Q  L  A  I  P  R  E  N  A  Q  I  S  L  D
      970                 990                1010
TGGAGATGTCACATTTTTTGGTGCATTGAAACTGCTGTGACCTACTTACACCATGTCTGT
 G  D  V  T  F  F  G  A  L  K  L  L
     1030                1050                1070
AGCTATTTTCCTCCCTTTCTCTGTACCTCTAAGAAGAAAGAATCTAACTGAAAATACCAA
     1090                1110                1130
AAAAAAAAAAAAAAAAAAAAAAAAAGTAGTTAAAAAAAAAAAAAAAAAAAAAAAAAAAA
     1150                1170
AAAAAAAAAAAAAAAAAAAAAACTCGGAGGGGG
```

FIG. 2A

```
        10                  30                  50
GAATTCGGCACGAGCTCCAAAGGCCTAGACCTTCAAAGTGCTCCTCGTGGAATGGATGAG
                                                  M  D  E
        70                  90                  110
TCTGCAAAGACCCTGCCACCACCGTGCCTCTGTTTTTGCTCCGAGAAAGGAGAAGATATG
S  A  K  T  L  P  P  P  C  L  C  F  C  S  E  K  G  E  D  M
       130                 150                 170
AAAGTGGGATATGATCCCATCACTCCGCAGAAGGAGGAGGGTGCCTGGTTTGGGATCTGC
K  V  G  Y  D  P  I  T  P  Q  K  E  E  G  A  W  F  G  I  C
       190                 210                 230
AGGGATGGAAGGCTGCTGGCTGCTACCCTCCTGCTGGCCCTGTTGTCCAGCAGTTTCACA
R  D  G  R  L  L  A  A  T  L  L  L  A  L  L  S  S  S  F  T
       250                 270                 290
GCGATGTCCTTGTACCAGTTGGCTGCCTTGCAAGCAGACCTGATGAACCTGCGCATGGAG
A  M  S  L  Y  Q  L  A  A  L  Q  A  D  L  M  N  L  R  M  E
       310                 330                 350
CTGCAGAGCTACCGAGGTTCAGCAACACCAGCCGCCGCGGGTGCTCCAGAGTTGACCGCT
L  Q  S  Y  R  G  S  A  T  P  A  A  A  G  A  P  E  L  T  A
       370                 390                 410
GGAGTCAAACTCCTGACACCGGCAGCTCCTCGACCCCACAACTCCAGCCGCGGCCACAGG
G  V  K  L  L  T  P  A  A  P  R  P  H  N  S  S  R  G  H  R
       430                 450                 470
AACAGACGCGCTTTCCAGGGACCAGAGGAAACAGAACAAGATGTAGACCTCTCAGCTCCT
N  R  R  A  F  Q  G  P  E  E  T  E  Q  D  V  D  L  S  A  P
       490                 510                 530
CCTGCACCATGCCTGCCTGGATGCCGCCATTCTCAACATGATGATAATGGAATGAACCTC
P  A  P  C  L  P  G  C  R  H  S  Q  H  D  D  N  G  M  N  L
       550                 570                 590
AGAAACATCATTCAAGACTGTCTGCAGCTGATTGCAGACAGCGACACGCCGACTATACGA
R  N  I  I  Q  D  C  L  Q  L  I  A  D  S  D  T  P  T  I  R
```

FIG. 2B

```
      610                 630                 650
AAAGGAACTTACACATTTGTTCCATGGCTTCTCAGCTTTAAAAGAGGAAATGCCTTGGAG
K  G  T  Y  T  F  V  P  W  L  L  S  F  K  R  G  N  A  L  E
      670                 690                 710
GAGAAAGAGAACAAAATAGTGGTGAGGCAAACAGGCTATTTCTTCATCTACAGCCAGGTT
E  K  E  N  K  I  V  V  R  Q  T  G  Y  F  F  I  Y  S  Q  V
      730                 750                 770
CTATACACGGACCCCATCTTTGCTATGGGTCATGTCATCCAGAGGAAGAAAGTACACGTC
L  Y  T  D  P  I  F  A  M  G  H  V  I  Q  R  K  K  V  H  V
      790                 810                 830
TTTGGGGACGAGCTGAGCCTGGTGACCCTGTTCCGATGTATTCAGAATATGCCCAAAACA
F  G  D  E  L  S  L  V  T  L  F  R  C  I  Q  N  M  P  K  T
      850                 870                 890
CTGCCCAACAATTCCTGCTACTCGGCTGGCATCGCGAGGCTGGAAGAAGGAGATGAGATT
L  P  N  N  S  C  Y  S  A  G  I  A  R  L  E  E  G  D  E  I
      910                 930                 950
CAGCTTGCAATTCCTCGGGAGAATGCACAGATTTCACGCAACGGAGACGACACCTTCTTT
Q  L  A  I  P  R  E  N  A  Q  I  S  R  N  G  D  D  T  F  F
      970                 990                1010
GGTGCCCTAAAACTGCTGTAACTCACTTGCTGGAGTGCGTGATCCCCTTCCCTCGTCTTC
G  A  L  K  L  L
     1030                1050                1070
TCTGTACCTCCGAGGGAGAAACAGACGACTGGAAAAACTAAAAGATGGGGAAAGCCGTCA
     1090                1110                1130
GCGAAAGTTTTCTCGTGACCCGTTGAATCTGATCCAAACCAGGAAATATAACAGACAGCC
     1150                1170                1190
```

FIG. 3A

```
          1                                                  50
Hagp3     MDDSTER.EQ SRLTSCLKKR EEMKLKECVS ILPRKESPSV RSSKDGKLLA
Magp3     MDESAKTLPP PCLCFCSEKG EDMKVGYDPI TPQKEEGAWF GICRDGRLLA
 cons     MD.S..... ..L..C..K. E.MK..... .....E.... ....DG.LLA

          51                                                 100
Hagp3     ATLLLALLSC CLTVVSFYQV AALQGDLASL RAELQGHHAE KLPAGAGAPK
Magp3     ATLLLALLSS SFTAMSLYQL AALQADLMNL RMELQSYRGS ATPAAAGAPE
 cons     ATLLLALLS. ..T..S.YQ. AALQ.DL..L R.ELQ..... ..PA.AGAP.

         101                                                 150
Hagp3     AGLEEAPAVT AGLKIFEPPA PGEGNSSQNS RNKRAVQGPE ET........
Magp3     ........LT AGVKLLTPAA PRPHNSSRGH RNRRAFQGPE ETEQDVDLSA
 cons     .........T AG.K...P.A P...NSS... RN.RA.QGPE ET........

          151                          B                  200
Hagp3     .......... .......... ...VTQDCLQ LIADSETPTI QKGSYTFVPW
Magp3     PPAPCLPGCR HSQHDDNGMN LRNIIQDCLQ LIADSDTPTI RKGTYTFVPW
 cons     .......... .......... .....QDCLQ LIADS.TPTI .KG.YTFVPW
```

FIG. 3B

```
        B'         C'        C          D                    E          250
Hagp3   LLSFKRGSAL EEKENKILVK ETGYFFIYGQ VLYTDKTYAM GHLIQRKKVH
Magp3   LLSFKRGNAL EEKENKIVVR QTGYFFIYSQ VLYTDPIFAM GHVIQRKKVH
 cons   LLSFKRG.AL EEKENKI.V. .TGYFFIY.Q VLYTD...AM GH.IQRKKVH

        251    F                     G                   H       300
Hagp3   VFGDELSLVT LFRCIQNMPE TLPNNSCYSA GIAKLEEGDE LQLAIPRENA
Magp3   VFGDELSLVT LFRCIQNMPK TLPNNSCYSA GIARLEEGDE IQLAIPRENA
 cons   VFGDELSLVT LFRCIQNMP. TLPNNSCYSA GIA.LEEGDE .QLAIPRENA

        301        I    317
Hagp3   QISLDGDVTF FGALKLL
Magp3   QISRNGDDTF FGALKLL
 cons   QIS..GD.TF FGALKLL
```

FIG. 4A

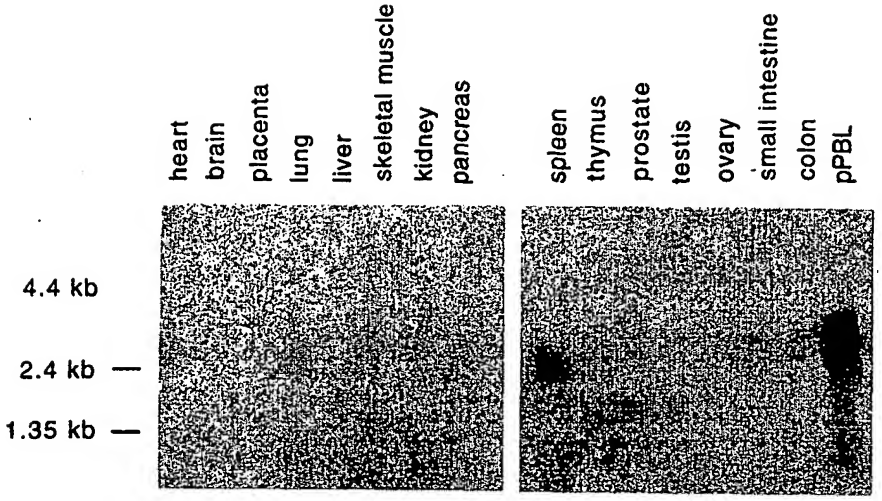

heart
brain
placenta
lung
liver
skeletal muscle
kidney
pancreas
spleen
thymus
prostate
testis
ovary
small intestine
colon
pPBL
4.4 kb
2.4 kb
1.35 kb

FIG. 4B

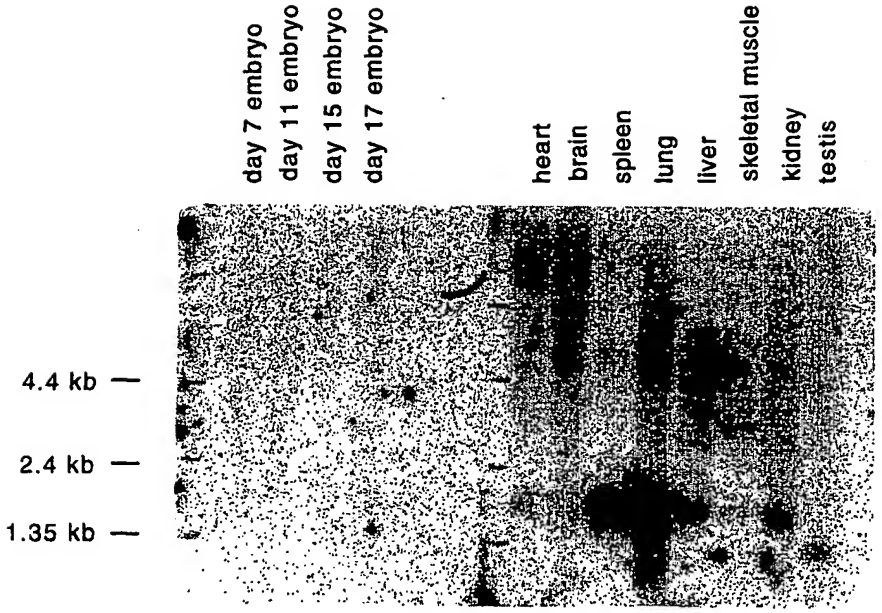

day 7 embryo
day 11 embryo
day 15 embryo
day 17 embryo
heart
brain
spleen
lung
liver
skeletal muscle
kidney
testis
4.4 kb
2.4 kb
1.35 kb

FIG. 5A

FIG. 5B

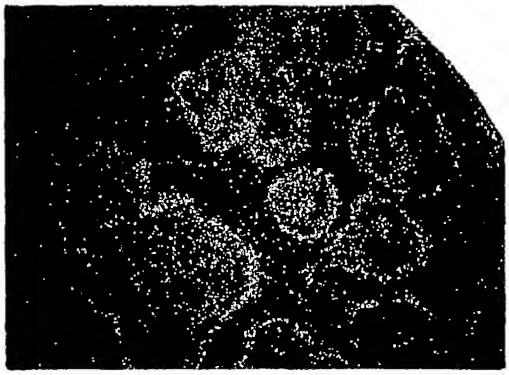


FIG. 5C

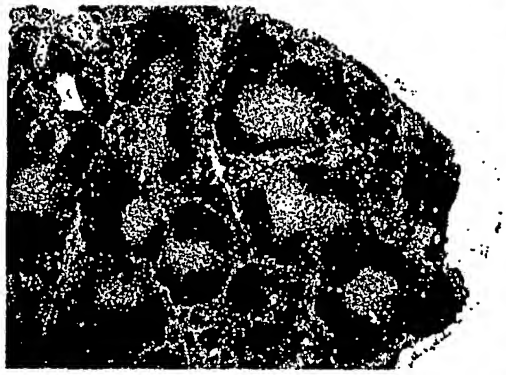


FIG. 5D

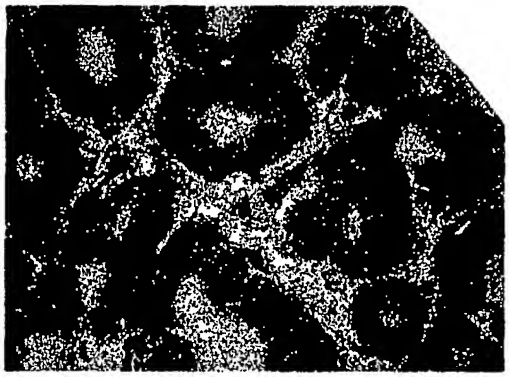


FIG. 5E

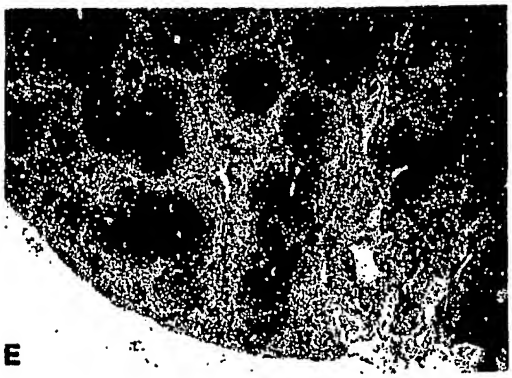


FIG. 5F

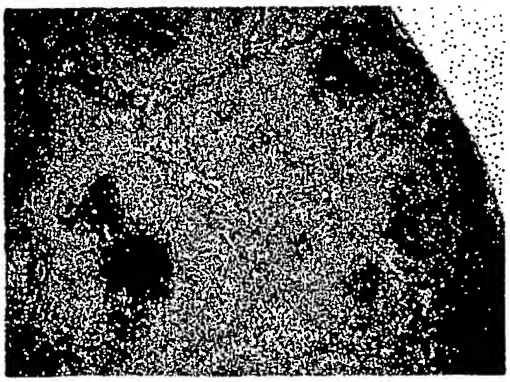


FIG. 6A

FIG. 6B

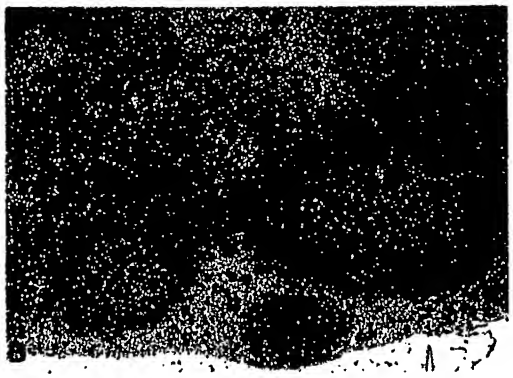


FIG. 6C

FIG. 6D

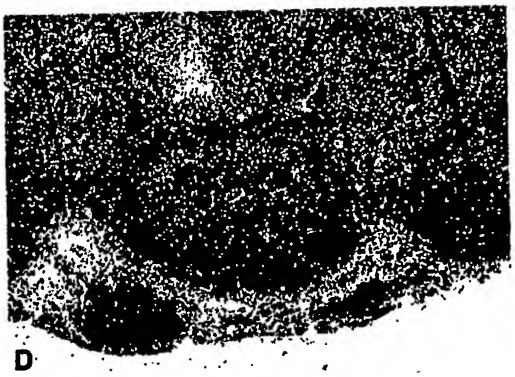


FIG. 6E

FIG. 6F

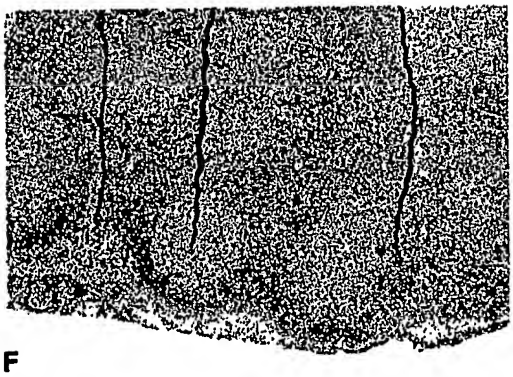
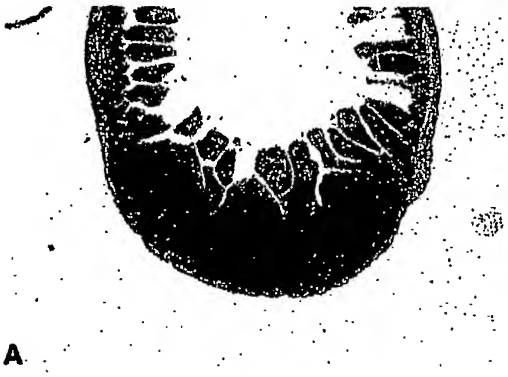


FIG. 7A



A

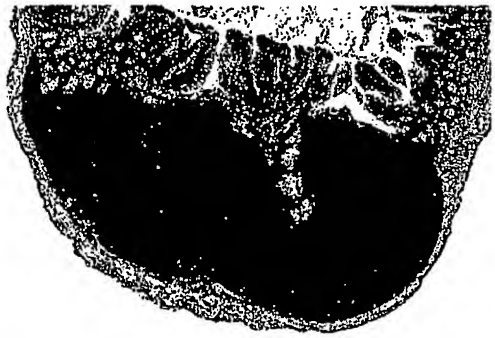
FIG. 7B

B

FIG. 7C

C

FIG. 7D



D

FIG. 7E

E

FIG. 7F

F

FIG. 8A

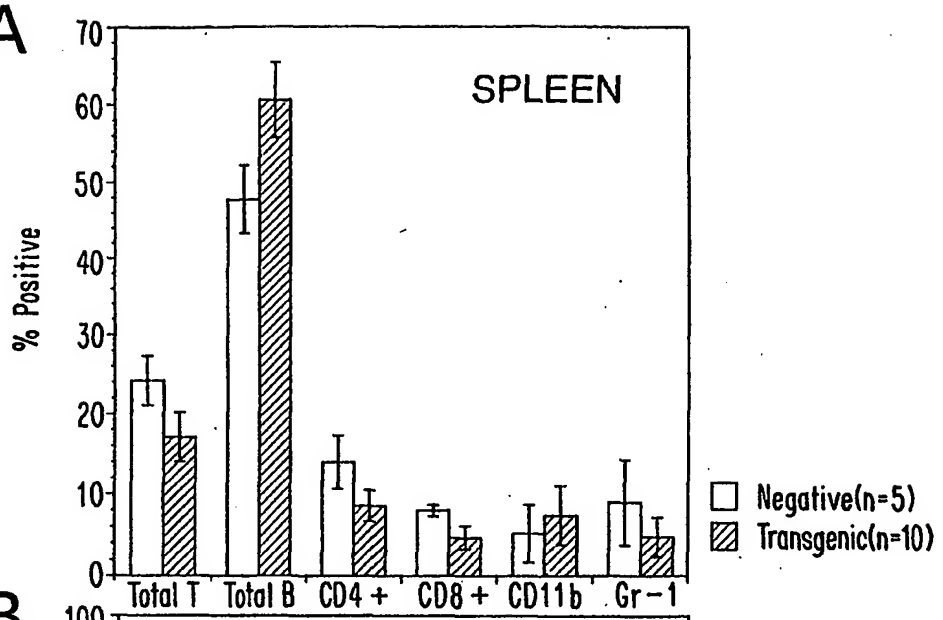


FIG. 8B

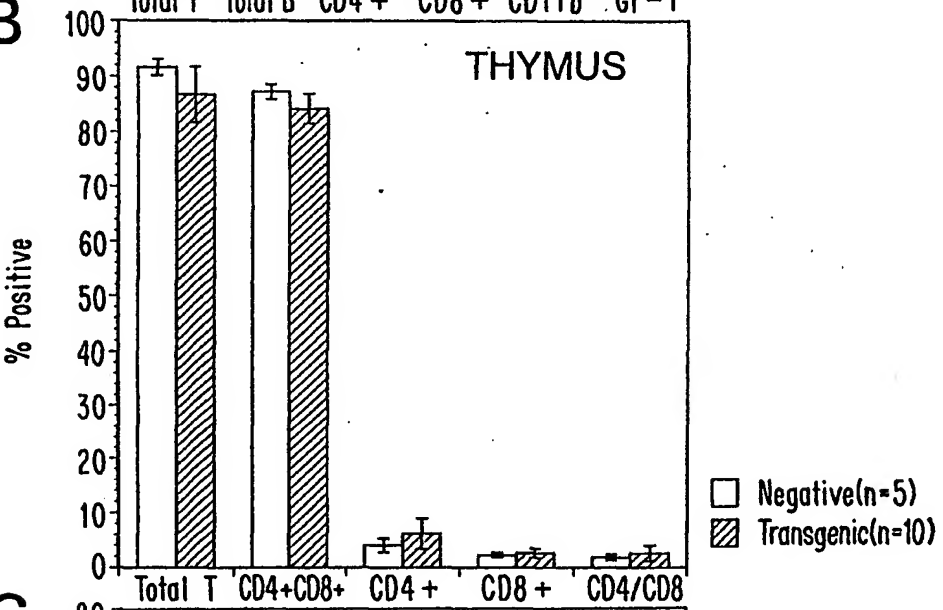


FIG. 8C

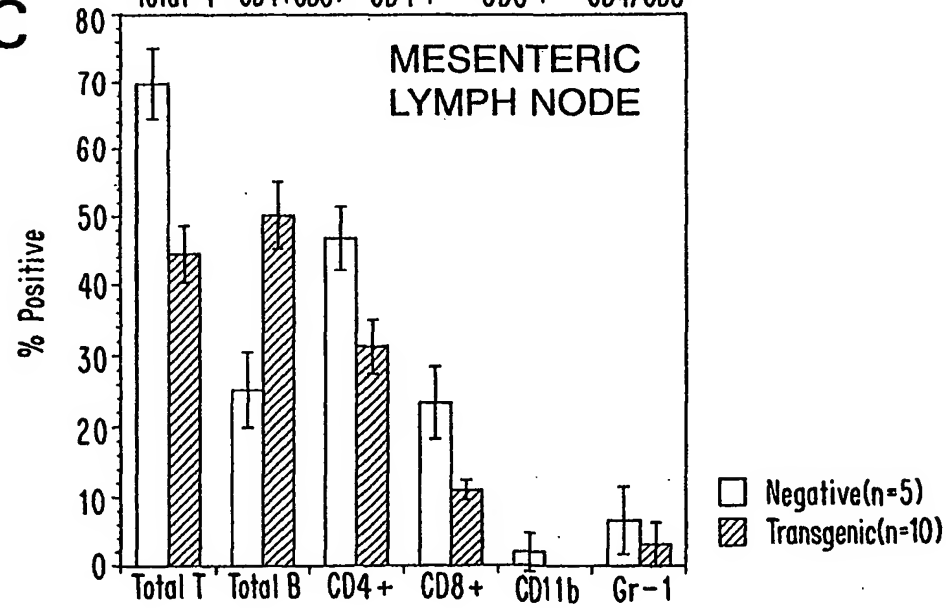


FIG. 9A

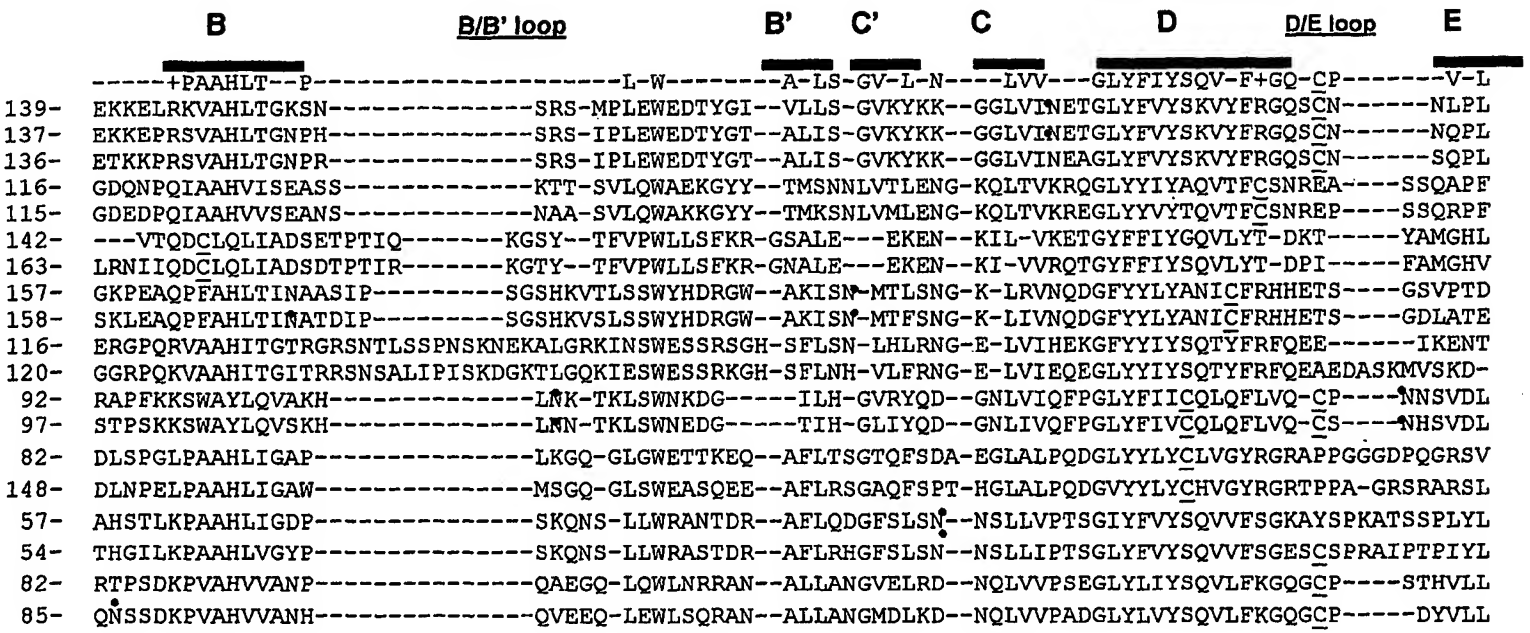
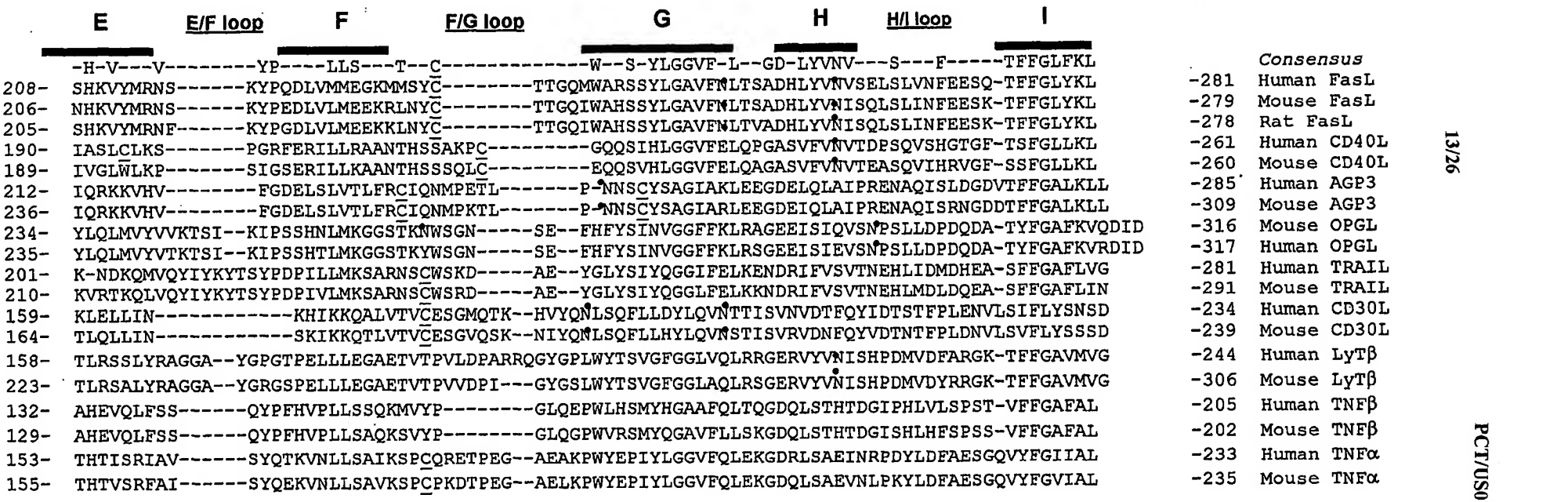

B
B/B' loop
B'
C'
C
D
D/E loop
E
-----+PAAHLT--P----------L-W--------A-LS-GV-L-N----LVV---GLYFIYSQV-F+GQ-CP-------V-L Consensus
139- EKKELRKVAHLTGKSN----------SRS-MPLEWEDTYGI--VLLS-GVKYKK--GGLVINETGLYFVYSKVYFRGQSCN------NLPL Human FasL
137- EKKEPRSVAHLTGNPH----------SRS-IPLEWEDTYGT--ALIS-GVKYKK--GGLVINETGLYFVYSKVYFRGQSCN------NQPL Mouse FasL
136- ETKKPRSVAHLTGNPR----------SRS-IPLEWEDTYGT--ALIS-GVKYKK--GGLVINEAGLYFVYSKVYFRGQSCN------SQPL Rat FasL
116- GDQNPQIAAHVISEASS----------KTT-SVLQWAEKGYY--TMSNNLVTLENG-KQLTVKRQGLYYIYAQVTFCSNREA----SSQAPF Human CD40L
115- GDEDPQIAAHVVSEANS----------NAA-SVLQWAKKGYY--TMKSNLVMLENG-KQLTVKREGLYYVYTQVTFCSNREP----SSQRPF Mouse CD40L
142- ---VTQDCLQLIADSETPTIQ-------KGSY--TFVPWLLSFKR-GSALE---EKEN--KIL-VKETGYFFIYGQVLYT-DKT-----YAMGHL Human AGP3
163- LRNIIQDCLQLIADSDTPTIR-------KGTY--TFVPWLLSFKR-GNALE---EKEN--KI-VVRQTGYFFIYSQVLYT-DPI-----FAMGHV Mouse AGP3
157- GKPEAQPFAHLTINAASIP---------SGSHKVTLSSWYHDRGW--AKISN-MTLSNG-K-LRVNQDGFYYLYANICFRHHETS----GSVPTD Mouse OPGL
158- SKLEAQPFAHLTINATDIP---------SGSHKVSLSSWYHDRGW--AKISN-MTFSNG-K-LIVNQDGFYYLYANICFRHHETS----GDLATE Human OPGL
116- ERGPQRVAAHITGTRGRSNTLSSPNSKNEKALGRKINSWESSRSGH-SFLSN-LHLRNG-E-LVIHEKGFYYIYSQTYFRFQEE------IKENT Human TRAIL
120- GGRPQKVAAHITGITRRSNSALIPISKDGKTLGQKIESWESSRKGH-SFLNH-VLFRNG-E-LVIEQEGLYYIYSQTYFRFQEAEDASKMVSKD- Mouse TRAIL
92- RAPFKKSWAYLQVAKH----------LNK-TKLSWNKDG-----ILH-GVRYQD--GNLVIQFPGLYFIICQLQFLVQ-CP----NNSVDL Human CD30L
97- STPSKKSWAYLQVSKH----------LNN-TKLSWNEDG-----TIH-GLIYQD--GNLIVQFPGLYFIVCQLQFLVQ-CS----NHSVDL Mouse CD30L
82- DLSPGLPAAHLIGAP----------LKGQ-GLGWETTKEQ--AFLTSGTQFSDA-EGLALPQDGLYYLYCLVGYRGRAPPGGGDPQGRSV Human LyTβ
148- DLNPELPAAHLIGAW----------MSGQ-GLSWEASQEE--AFLRSGAQFSPT-HGLALPQDGVYYLYCHVGYRGRTPPA-GRSRARSL Mouse LyTβ
57- AHSTLKPAAHLIGDP----------SKQNS-LLWRANTDR--AFLQDGFSLSN--NSLLVPTSGIYFVYSQVVFSGKAYSPKATSSPLYL Human TNFβ
54- THGILKPAAHLVGYP----------SKQNS-LLWRASTDR--AFLRHGFSLSN--NSLLIPTSGLYFVYSQVVFSGESCSPRAIPTPIYL Mouse TNFβ
82- RTPSDKPVAHVVANP----------QAEGQ-LQWLNRRAN--ALLANGVELRD--NQLVVPSEGLYLIYSQVLFKGQGCP----STHVLL Human TNFα
85- QNSSDKPVAHVVANH----------QVEEQ-LEWLSQRAN--ALLANGMDLKD--NQLVVPADGLYLVYSQVLFKGQGCP-----DYVLL Mouse TNFα

FIG. 9B


E
E/F loop
F
F/G loop
G
H
H/I loop
I
-H-V---V--------YP----LLS---T--C----------W--S-YLGGVF-L--GD-LYVNV---S---F-----TFFGLFKL
208- SHKVYMRNS------KYPQDLVMMEGKMMSYC--------TTGQMWARSSYLGAVFNLTSADHLYVNVSELSLVNFEESQ-TFFGLYKL
206- NHKVYMRNS------KYPEDLVLMEEKRLNYC--------TTGQIWAHSSYLGAVFNLTSADHLYVNISQLSLINFEESK-TFFGLYKL
205- SHKVYMRNF------KYPGDLVLMEEKKLNYC--------TTGQIWAHSSYLGAVFNLTVADHLYVNISQLSLINFEESK-TFFGLYKL
190- IASLCLKS-------PGRFERILLRAANTHSSAKPC---------GQQSIHLGGVFELQPGASVFVNVTDPSQVSHGTGF-TSFGLLKL
189- IVGLWLKP-------SIGSERILLKAANTHSSSQLC---------EQQSVHLGGVFELQAGASVFVNVTEASQVIHRVGF-SSFGLLKL
212- IQRKKVHV--------FGDELSLVTLFRCIQNMPETL-------P-NNSCYSAGIAKLEEGDELQLAIPRENAQISLDGDVTFFGALKLL
236- IQRKKVHV--------FGDELSLVTLFRCIQNMPKTL-------P-NNSCYSAGIARLEEGDEIQLAIPRENAQISRNGDDTFFGALKLL
234- YLQLMVYVVKTSI--KIPSSHNLMKGGSTKNWSGN-----SE--FHFYSINVGGFFKLRAGEEISIQVSNPSLLDPDQDA-TYFGAFKVQDID
235- YLQLMVYVTKTSI--KIPSSHTLMKGGSTKYWSGN-----SE--FHFYSINVGGFFKLRSGEEISIEVSNPSLLDPDQDA-TYFGAFKVRDID
201- K-NDKQMVQYIYKYTSYPDPILLMKSARNSCWSKD-----AE--YGLYSIYQGGIFELKENDRIFVSVTNEHLIDMDHEA-SFFGAFLVG
210- KVRTKQLVQYIYKYTSYPDPIVLMKSARNSCWSRD-----AE--YGLYSIYQGGLFELKKNDRIFVSVTNEHLMDLDQEA-SFFGAFLIN
159- KLELLIN-----------KHIKKQALVTVCESGMQTK--HVYQNLSQFLLDYLQVNTTISVNVDTFQYIDTSTFPLENVLSIFLYSNSD
164- TLQLLIN-----------SKIKKQTLVTVCESGVQSK--NIYQNLSQFLLHYLQVNSTISVRVDNFQYVDTNTFPLDNVLSVFLYSSSD
158- TLRSSLYRAGGA--YGPGTPELLLEGAETVTPVLDPARRQGYGPLWYTSVGFGGLVQLRRGERVYVNISHPDMVDFARGK-TFFGAVMVG
223- TLRSALYRAGGA--YGRGSPELLLEGAETVTPVVDPI---GYGSLWYTSVGFGGLAQLRSGERVYVNISHPDMVDYRRGK-TFFGAVMVG
132- AHEVQLFSS------QYPFHVPLLSSQKMVYP--------GLQEPWLHSMYHGAAFQLTQGDQLSTHTDGIPHLVLSPST-VFFGAFAL
129- AHEVQLFSS------QYPFHVPLLSAQKSVYP--------GLQGPWVRSMYQGAVFLLSKGDQLSTHTDGISHLHFSPSS-VFFGAFAL
153- THTISRIAV------SYQTKVNLLSAIKSPCQRETPEG--AEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLDFAESGQVYFGIIAL
155- THTVSRFAI------SYQEKVNLLSAVKSPCPKDTPEG--AELKPWYEPIYLGGVFQLEKGDQLSAEVNLPKYLDFAESGQVYFGVIAL
Consensus
-281 Human FasL
-279 Mouse FasL
-278 Rat FasL
-261 Human CD40L
-260 Mouse CD40L
-285 Human AGP3
-309 Mouse AGP3
-316 Mouse OPGL
-317 Human OPGL
-281 Human TRAIL
-291 Mouse TRAIL
-234 Human CD30L
-239 Mouse CD30L
-244 Human LyTβ
-306 Mouse LyTβ
-205 Human TNFβ
-202 Mouse TNFβ
-233 Human TNFα
-235 Mouse TNFα

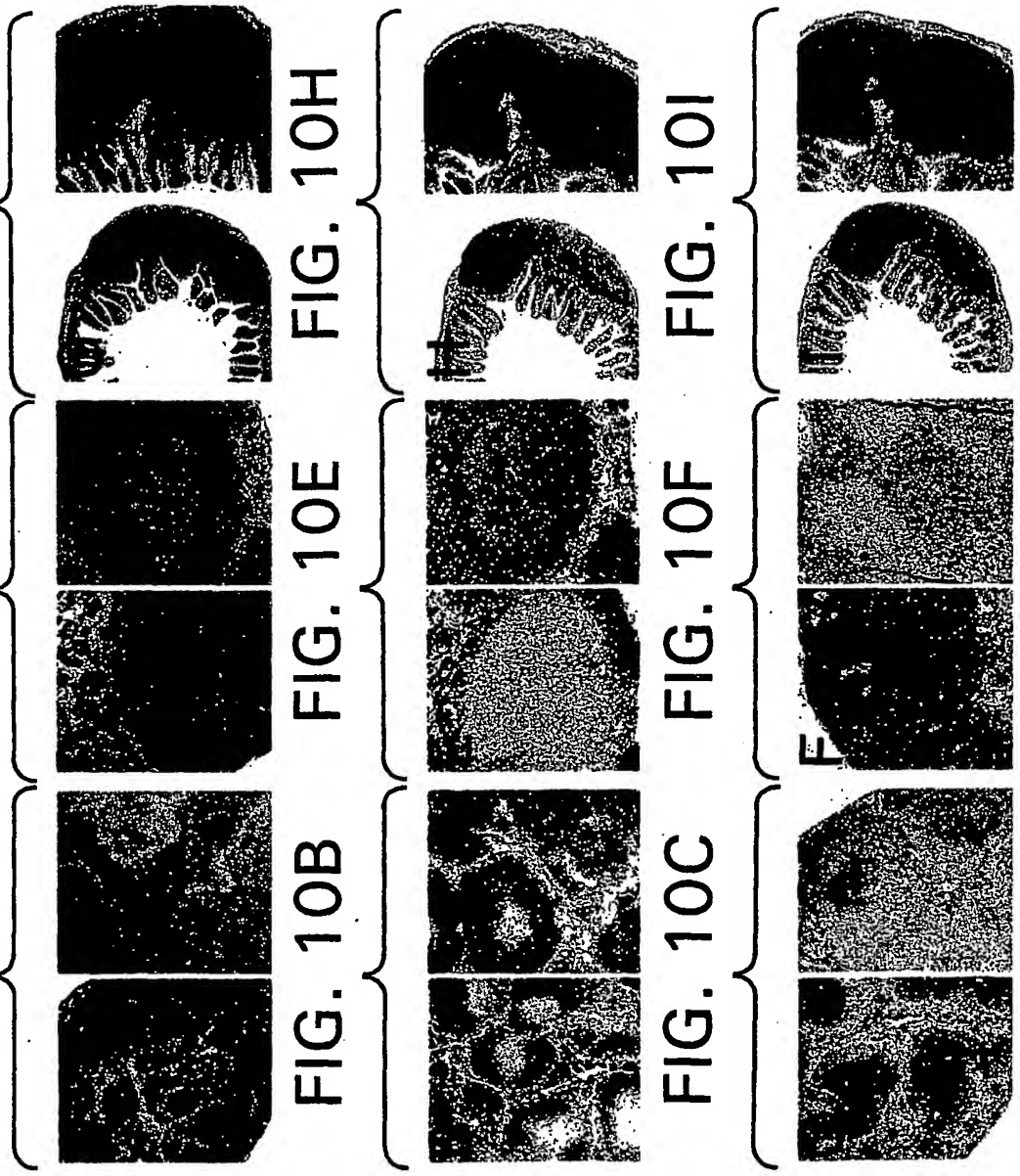


FIG. 10A
FIG. 10D
FIG. 10G
FIG. 10B
FIG. 10E
FIG. 10H
FIG. 10C
FIG. 10F
FIG. 10I

FIG. 11A

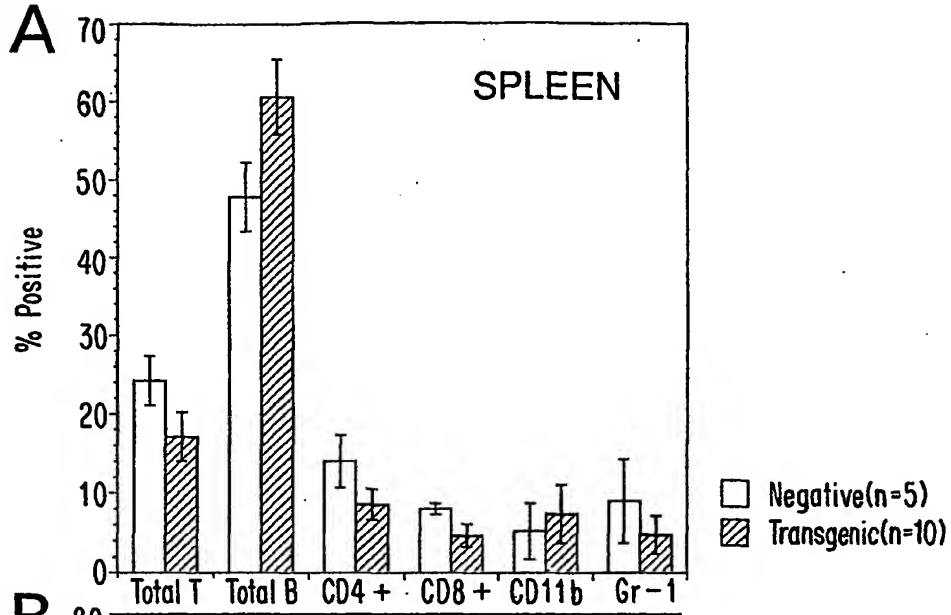


FIG. 11B

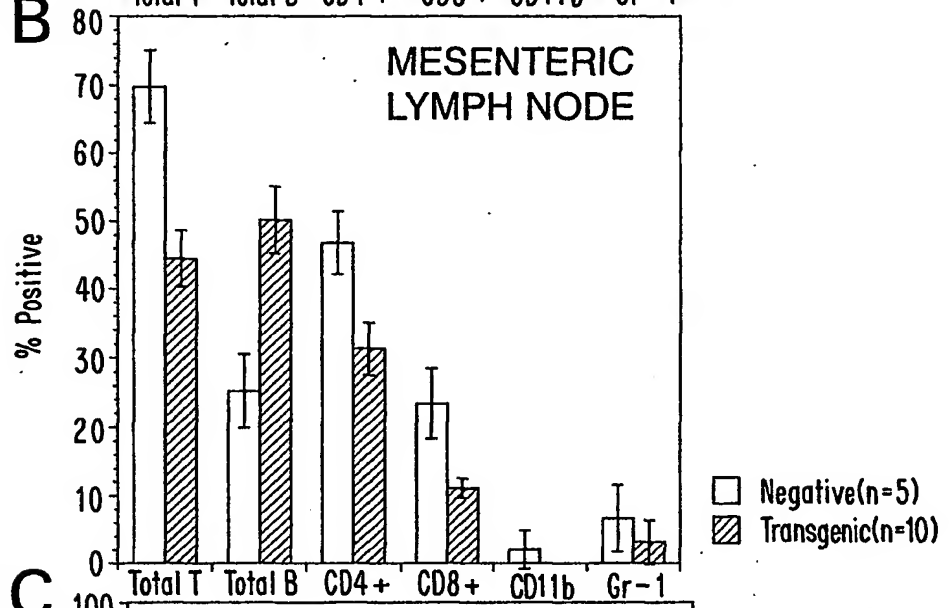


FIG. 11C

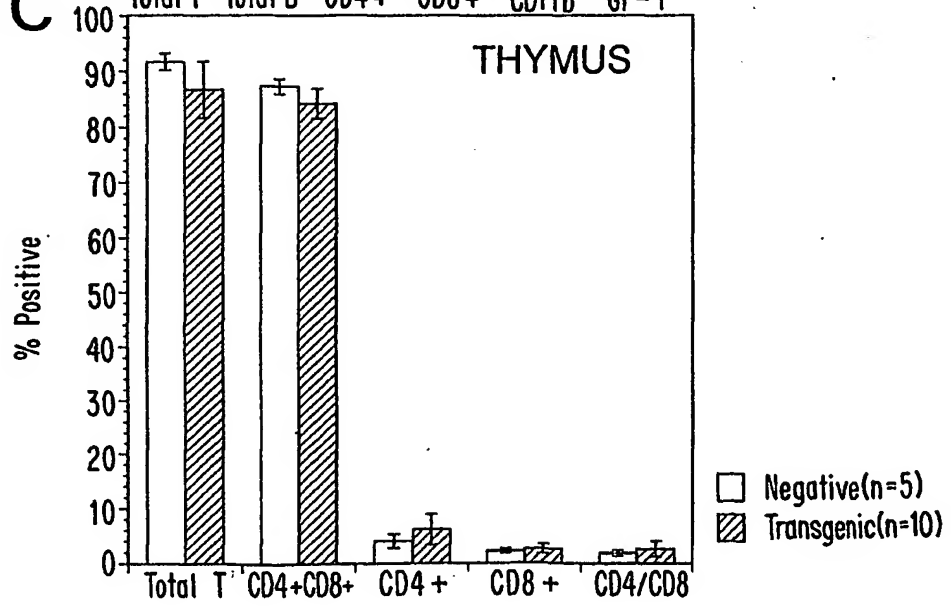


FIG. 12A

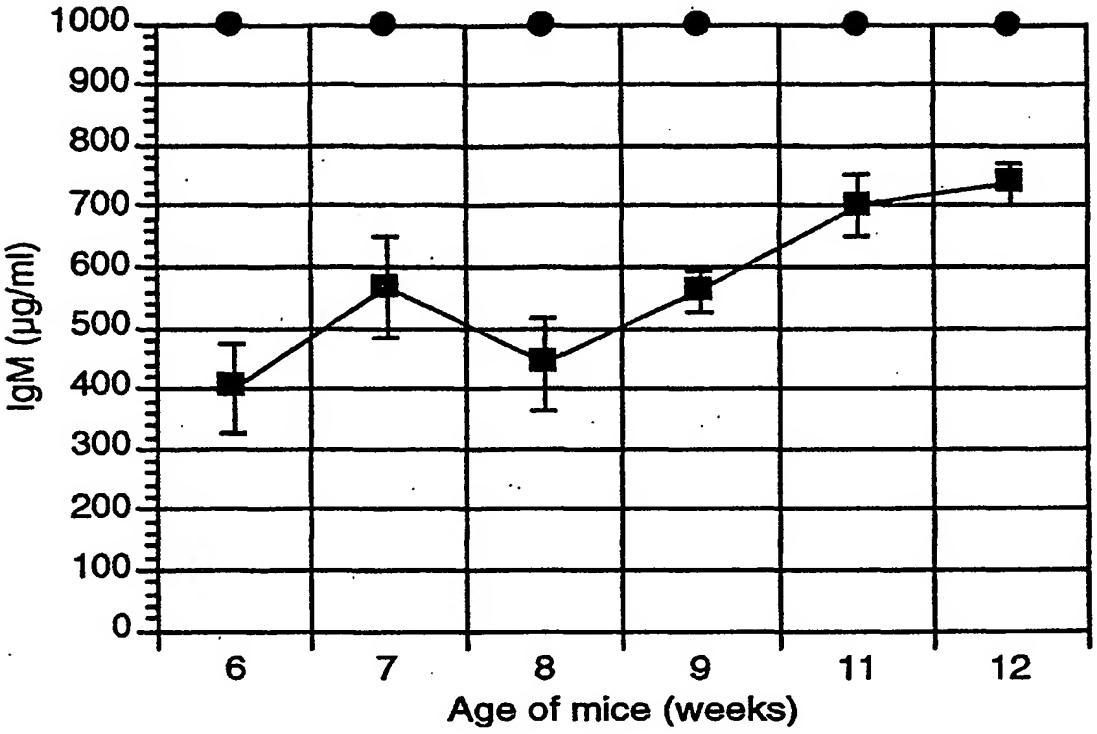


FIG. 12B

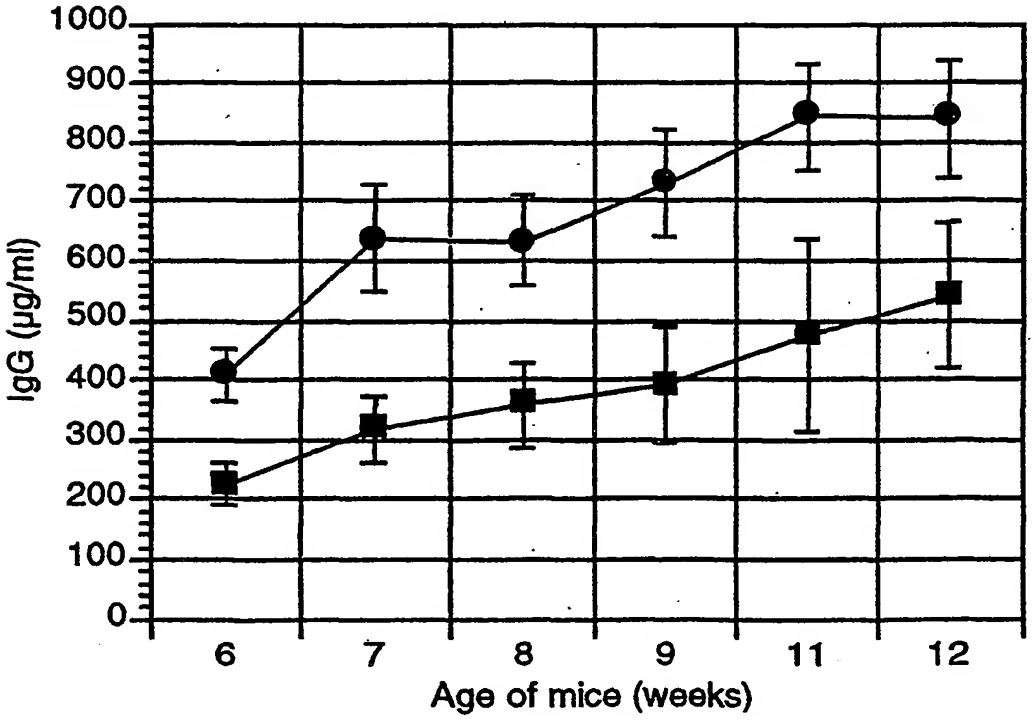


FIG. 12C

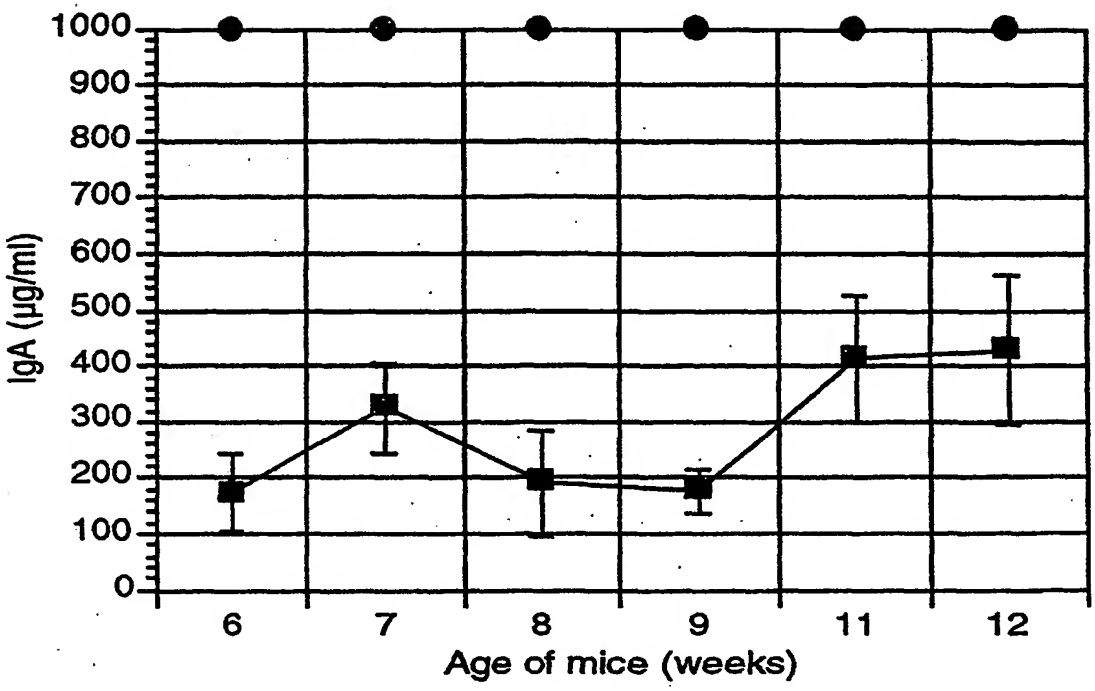
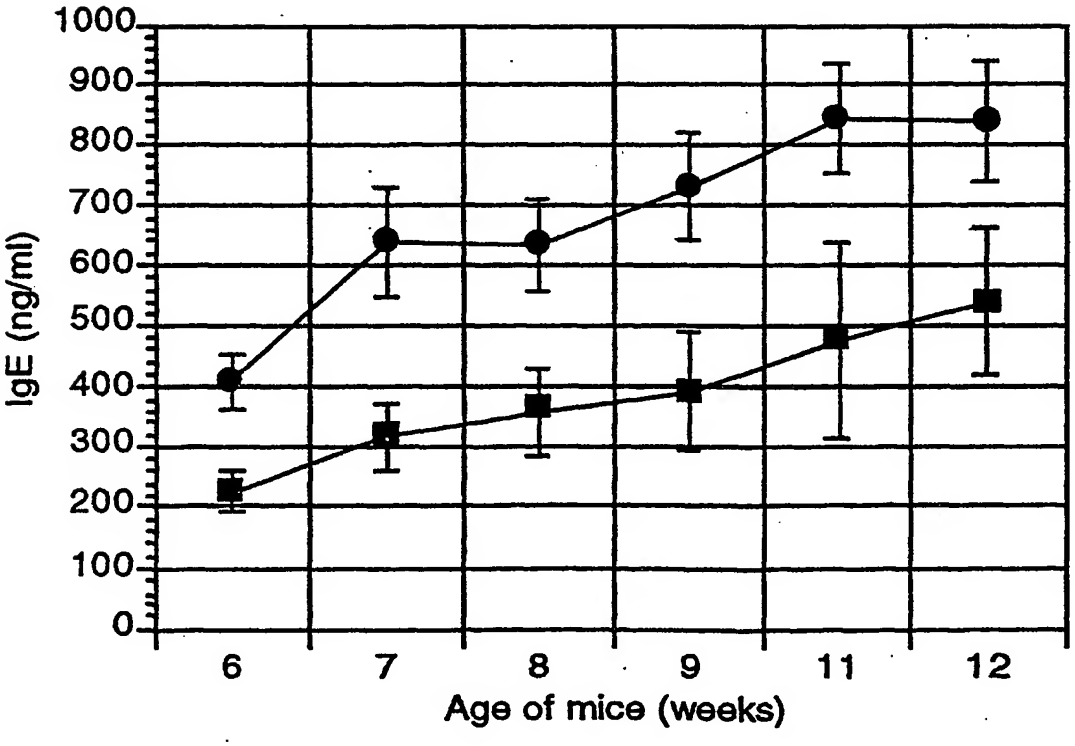


FIG. 12D



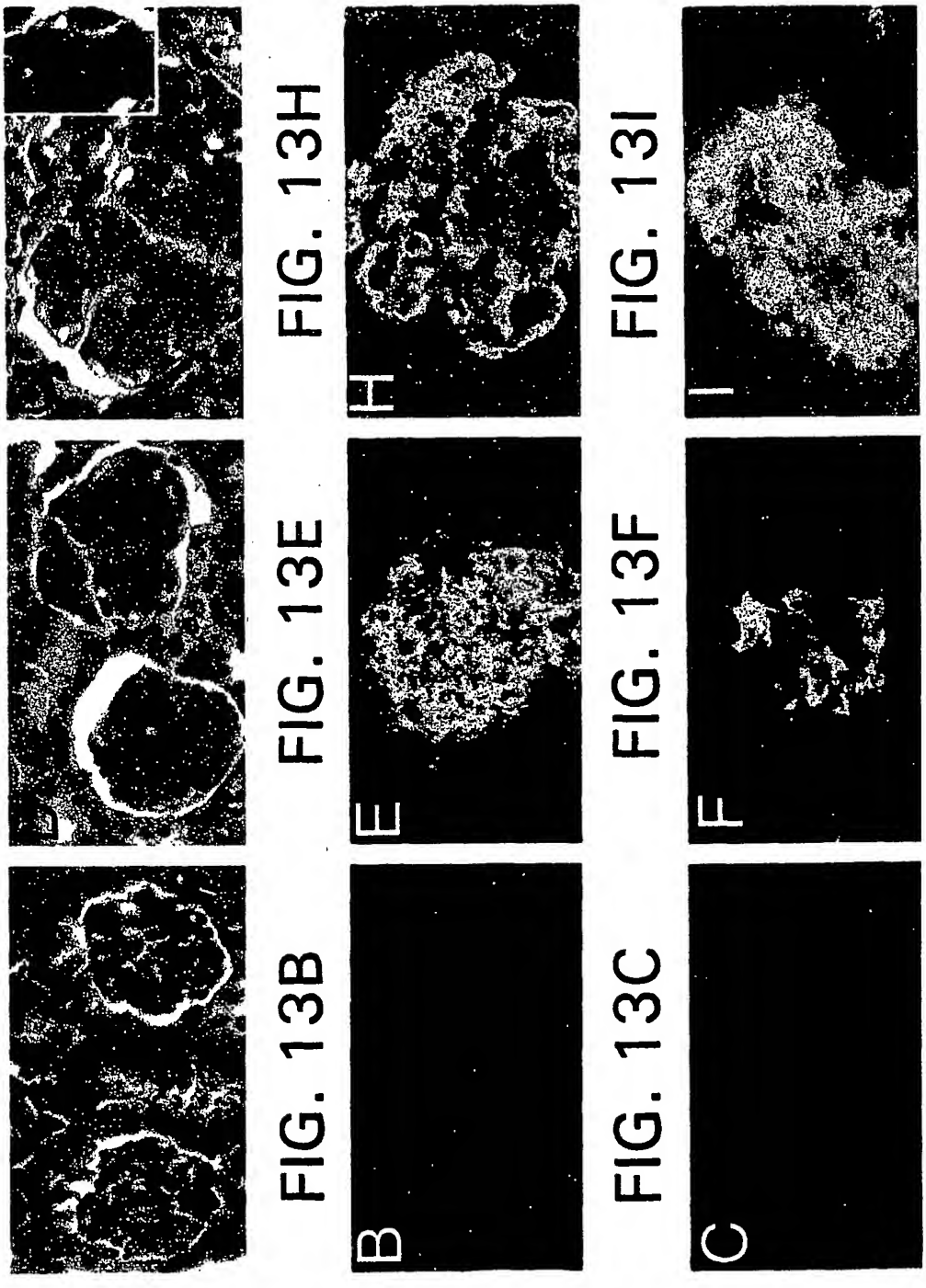


FIG. 13A
FIG. 13B
FIG. 13C
FIG. 13D
FIG. 13E
FIG. 13F
FIG. 13G
FIG. 13H
FIG. 13I
B
C
E
F
H
I

FIG. 14A

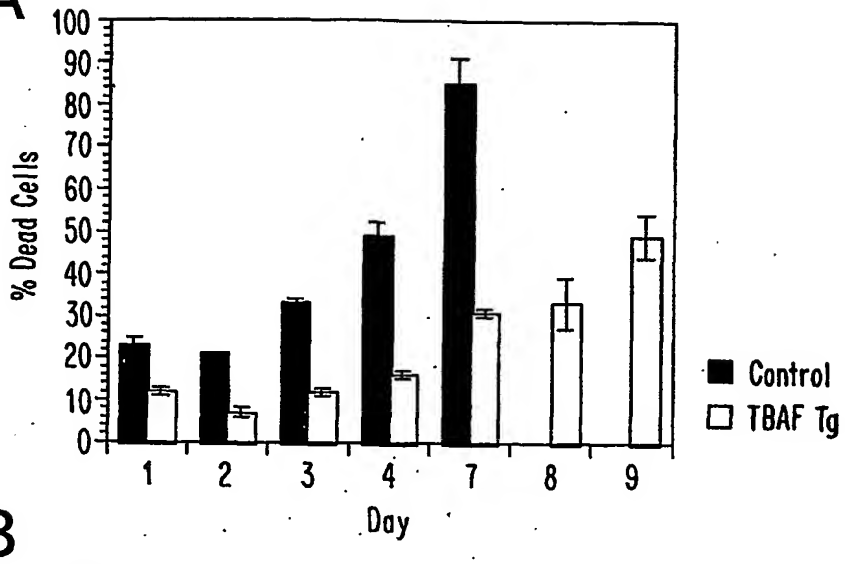


FIG. 14B

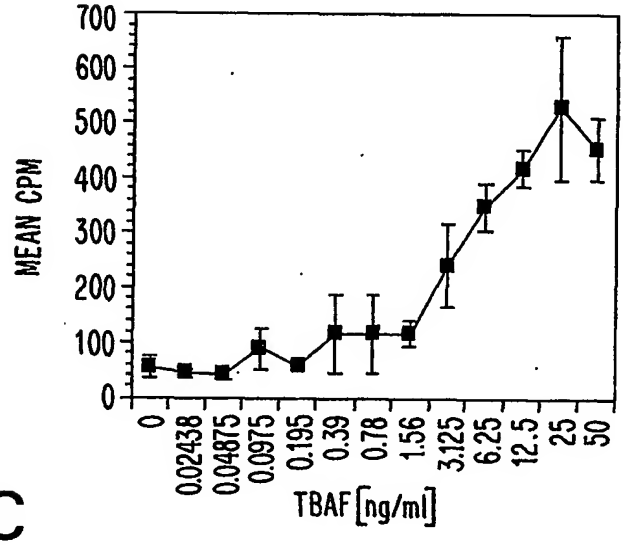


FIG. 14C

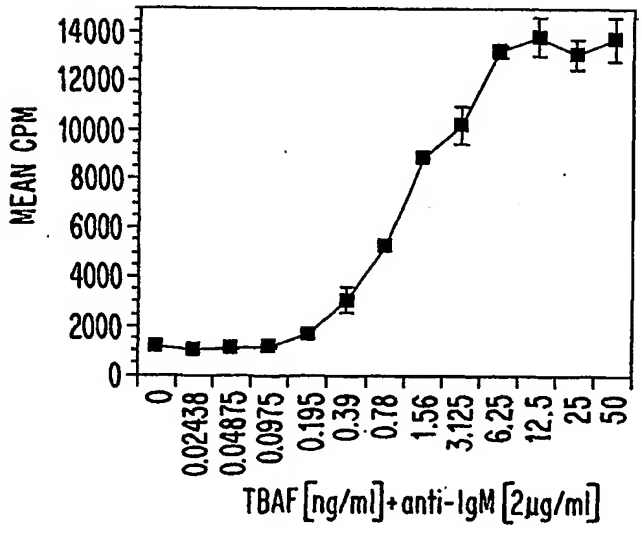


FIG. 15

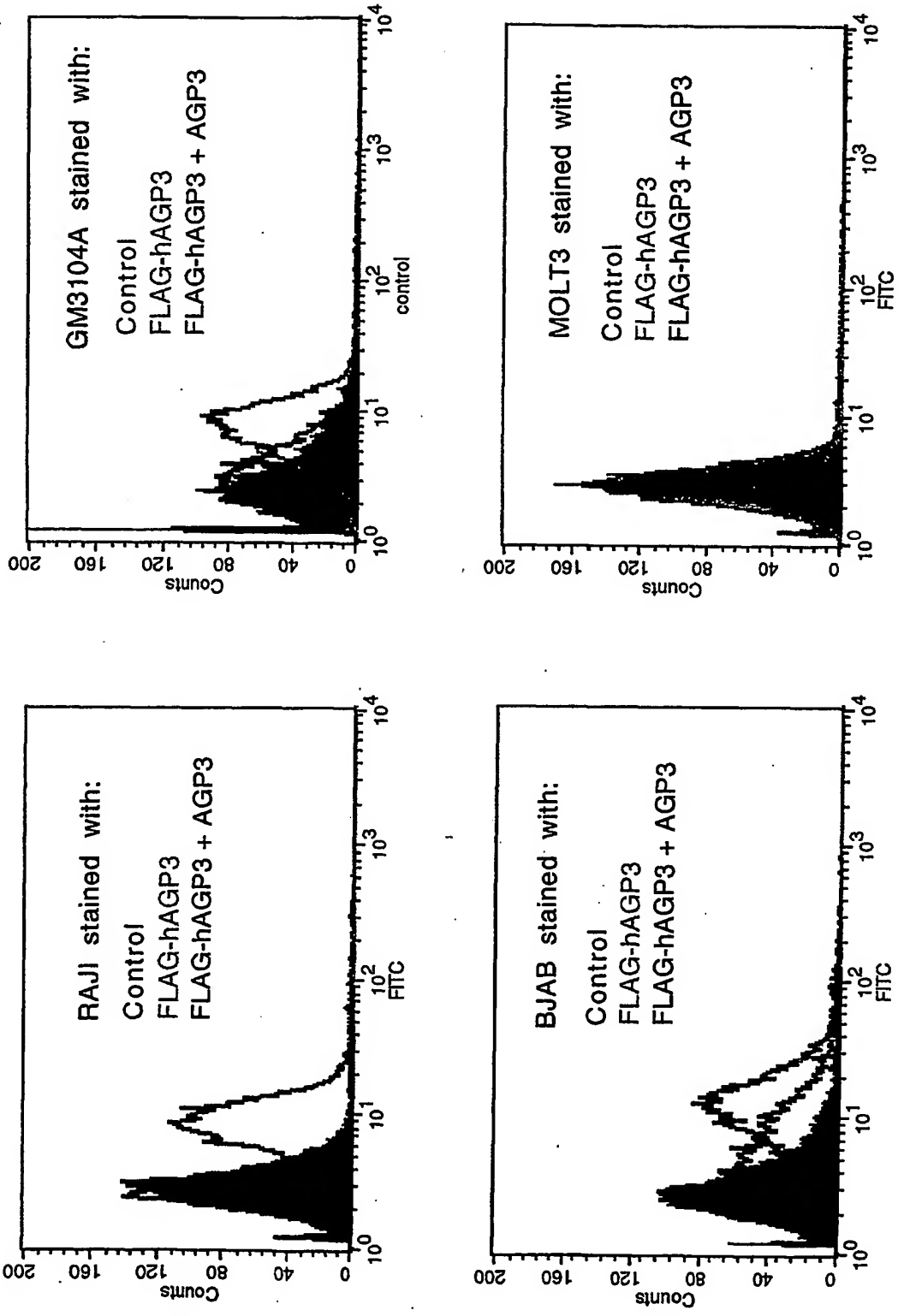

GM3104A stained with:
Control
FLAG-hAGP3
FLAG-hAGP3 + AGP3
Counts
control
MOLT3 stained with:
Control
FLAG-hAGP3
FLAG-hAGP3 + AGP3
Counts
FITC
RAJI stained with:
Control
FLAG-hAGP3
FLAG-hAGP3 + AGP3
Counts
FITC
BJAB stained with:
Control
FLAG-hAGP3
FLAG-hAGP3 + AGP3
Counts
FITC

FIG. 16

Alignment of AGP3-binding pools 13B4 and 13H11
N-terminal sequence

```
  1 GTCGACCCACGCGTCCG.......ATCCTGAGTAATGAGTGGCCTGGGCC 43
    |||||||||||||||||       |||||||||||||||||||||||||
  1 GTCGACCCACGCGTCCGAATAAGCATCCTGAGTAATGAGTGGCCTGGGCC 50

 44 GGAGCAGGCGAGGTGGCCGGAGCCGTGTGGACCAGGAGGAGCGCTTTCCA 93
    ||||||||||||||||||||||||||||||||||||||||||||||||||
 51 GGAGCAGGCGAGGTGGCCGGAGCCGTGTGGACCAGGAGGAGCGCTTTCCA 100

 94 CAGGGCCTGTGGACAGGGGTGGCTATGAGATCCTGCCCCGAAGAGCAGTA 143
    ||||||||||||||||||||||||||||||||||||||||||||||||||
101 CAGGGCCTGTGGACAGGGGTGGCTATGAGATCCTGCCCCGAAGAGCAGTA 150

144 CTGGGATCCTCTGCTGGGTACCTGCATGTCCTGCAAAACCATTTGCAACC 193
    ||||||||||||||||||||||||||||||||||||||||||||||||||
151 CTGGGATCCTCTGCTGGGTACCTGCATGTCCTGCAAAACCATTTGCAACC 200

194 ATCAGAGCCAGCGCACCTGTGCAGCCTTCTGCAGGTCACTCAGCTGCCGC 243
    ||||||||||||||||||||||||||||||||||||||||||||||||||
201 ATCAGAGCCAGCGCACCTGTGCAGCCTTCTGCAGGTCACTCAGCTGCCGC 250

244 AAGGAGCAAGGCAAGTTCTATGACCATCTCCTGAGGGACTGCATCAGCTG 293
    ||||||||||||||||||||||||||||||||||||||||||||||||||
251 AAGGAGCAAGGCAAGTTCTATGACCATCTCCTGAGGGACTGCATCAGCTG 300

294 TGCCTCCATCTGTGGACAGCACCCTAAGCAATGTGCATACTTCTGTGAGA 343
    ||||||||||||||||||||||||||||||||||||||||||||||||||
301 TGCCTCCATCTGTGGACAGCACCCTAAGCAATGTGCATACTTCTGTGAGA 350

344 ACAAGCTCAGGAGCCCAGTGAACCTTCCACCAGAGCTCAGGAGACAGCGG 393
    ||||||||||||||||||||||||||||||||||||||||||||||||||
351 ACAAGCTCAGGAGCCCAGTGAACCTTCCACCAGAGCTCAGGAGACAGCGG 400
```

FIG. 17

Human AGP3 receptor sequence

```
GTCGACCCACGCGTCCGATCCTGAGTAATGAGTGGCCTGGGCCGGAGCAGGCGAGGTGGC
                              M  S  G  L  G  R  S  R  R  G  G
CGGAGCCGTGTGGACCAGGAGGAGCGCTTTCCACAGGGCCTGTGGACAGGGGTGGCTATG
R  S  R  V  D  Q  E  E  R  F  P  Q  G  L  W  T  G  V  A  M
AGATCCTGCCCCGAAGAGCAGTACTGGGATCCTCTGCTGGGTACCTGCATGTCCTGCAAA
R  S  C  P  E  E  Q  Y  W  D  P  L  L  G  T  C  M  S  C  K
ACCATTTGCAACCATCAGAGCCAGCGCACCTGTGCAGCCTTCTGCAGGTCACTCAGCTGC
T  I  C  N  H  Q  S  Q  R  T  C  A  A  F  C  R  S  L  S  C
CGCAAGGAGCAAGGCAAGTTCTATGACCATCTCCTGAGGGACTGCATCAGCTGTGCCTCC
R  K  E  Q  G  K  F  Y  D  H  L  L  R  D  C  I  S  C  A  S
ATCTGTGGACAGCACCCTAAGCAATGTGCATACTTCTGTGAGAACAAGCTCAGGAGCCCA
I  C  G  Q  H  P  K  Q  C  A  Y  F  C  E  N  K  L  R  S  P
GTGAACCTTCCACCAGAGCTCAGGAGACAGCGGAGTGGAGAAGTTGAAAACAATTCAGAC
V  N  L  P  P  E  L  R  R  Q  R  S  G  E  V  E  N  N  S  D
AACTCGGGAAGGTACCAAGGACTGGAGCACAGAGGCTCAGAAGCAAGTCCAGCTCTCCCG
N  S  G  R  Y  Q  G  L  E  H  R  G  S  E  A  S  P  A  L  P
GGGCTGAAGCTGAGTGCAGATCAGGTGGCCCTGGTCTACAGCACGCTGGGGCTCTGCCTG
G  L  K  L  S  A  D  Q  V  A  L  V  Y  S  T  L  G  L  C  L
TGTGCCGTCCTCTGCTGCTTCCTGGTGGCGGTGGCCTGCTTCCTCAAGATGAGGGGGGAT
C  A  V  L  C  C  F  L  V  A  V  A  C  F  L  K  M  R  G  D
CCCTGCTCCTGCCAGCCCCGCTCAAGGCCCCGTCAAAGTCCGGCCAAGTCTTCCCAGGAT
P  C  S  C  Q  P  R  S  R  P  R  Q  S  P  A  K  S  S  Q  D
CACGCGATGGAAGCCGGCAGCCCTGTGAGCACATCCCCCGAGCCAGTGGAGACCTGCAGC
H  A  M  E  A  G  S  P  V  S  T  S  P  E  P  V  E  T  C  S
TTCTGCTTCCCTGAGTGCAGGGCGCCCACGCAGGAGAGCGCAGTCACGCCTGGGACCCCC
F  C  F  P  E  C  R  A  P  T  Q  E  S  A  V  T  P  G  T  P
GACCCCACTTGTGCTGGAAGGTGGGGGTGCCACACCAGGACCACAGTCCTGCAGCCTTGC
D  P  T  C  A  G  R  W  G  C  H  T  R  T  T  V  L  Q  P  C
CCACACATCCCAGACAGCGGCCTTGGCATTGTGTGTGTGCCTGCCCAGGAGGGGGGCCCA
P  H  I  P  D  S  G  L  G  I  V  C  V  P  A  Q  E  G  G  P
GGTGCATAAATGGGGGTCAGGGAGGGAAAGGAGGAGGGAGAGAGATGGAGAGGAGGGGAG
G  A
AGAGAAAGAGAGGTGGGGAGAGGGGAGAGAGATATGAGGAGAGAGAGACAGAGGAGGCAG
AGAGGGAGAGAAACAGAGGAGACAGAGAGGGAGAGAGAGACAGAGGGAGAGAGAGACAGA
GAGGAAGAGAGGCAGAGAGGGAAAGAGGCAGAGAAGGAAAGAGACAGGCAGAGAAGGAGA
GAGGCAGAGAGGGAGAGAGGCAGAGAGGGAGAGAGGCAGAGAGACAGAGAGGGAGAGAGG
GACAGAGAGAGATAGAGCAGGAGGTCGGGGCACTCTGAGTCCCAGTTCCCAGTGCAGCTG
TAGGTCGTCATCACCTAACCACACGTGCAATAAAGTCCTCGTGCCTGCTGCTCACAGCCC
CCGAGAGCCCCTCCTCCTGG
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FIG. 18

AGP3 receptor protein structure

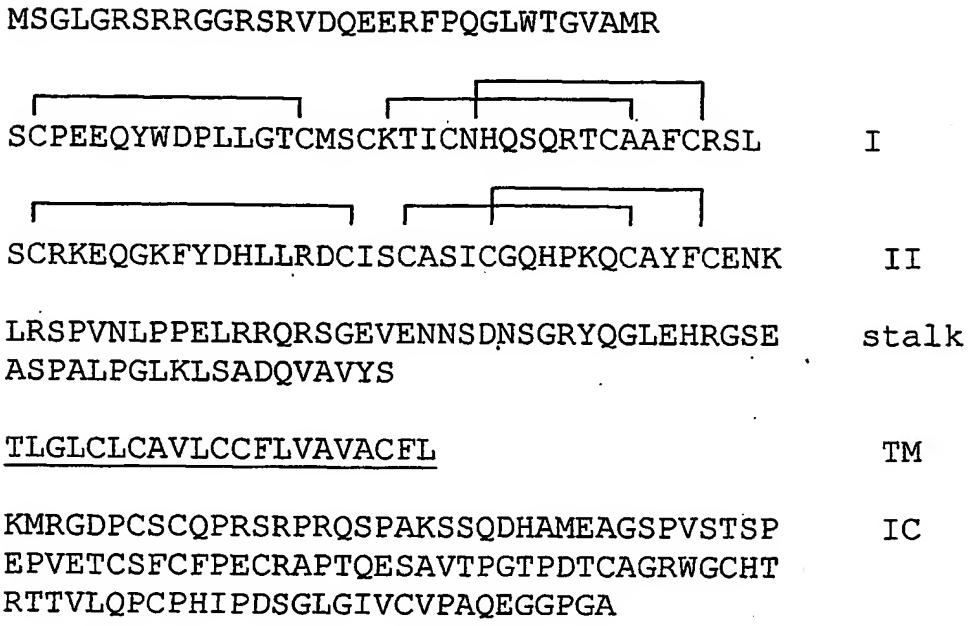


FIG. 19

Alignment of AGP3 receptor and TNFR1 extracellular domain

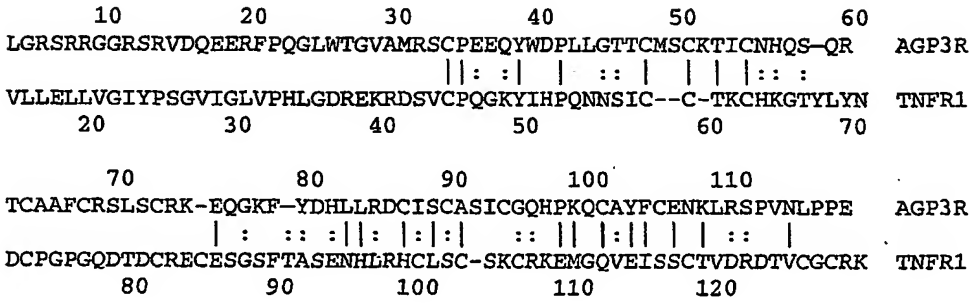


FIG. 20

Human AGP3 receptor mRNA tissue distribution

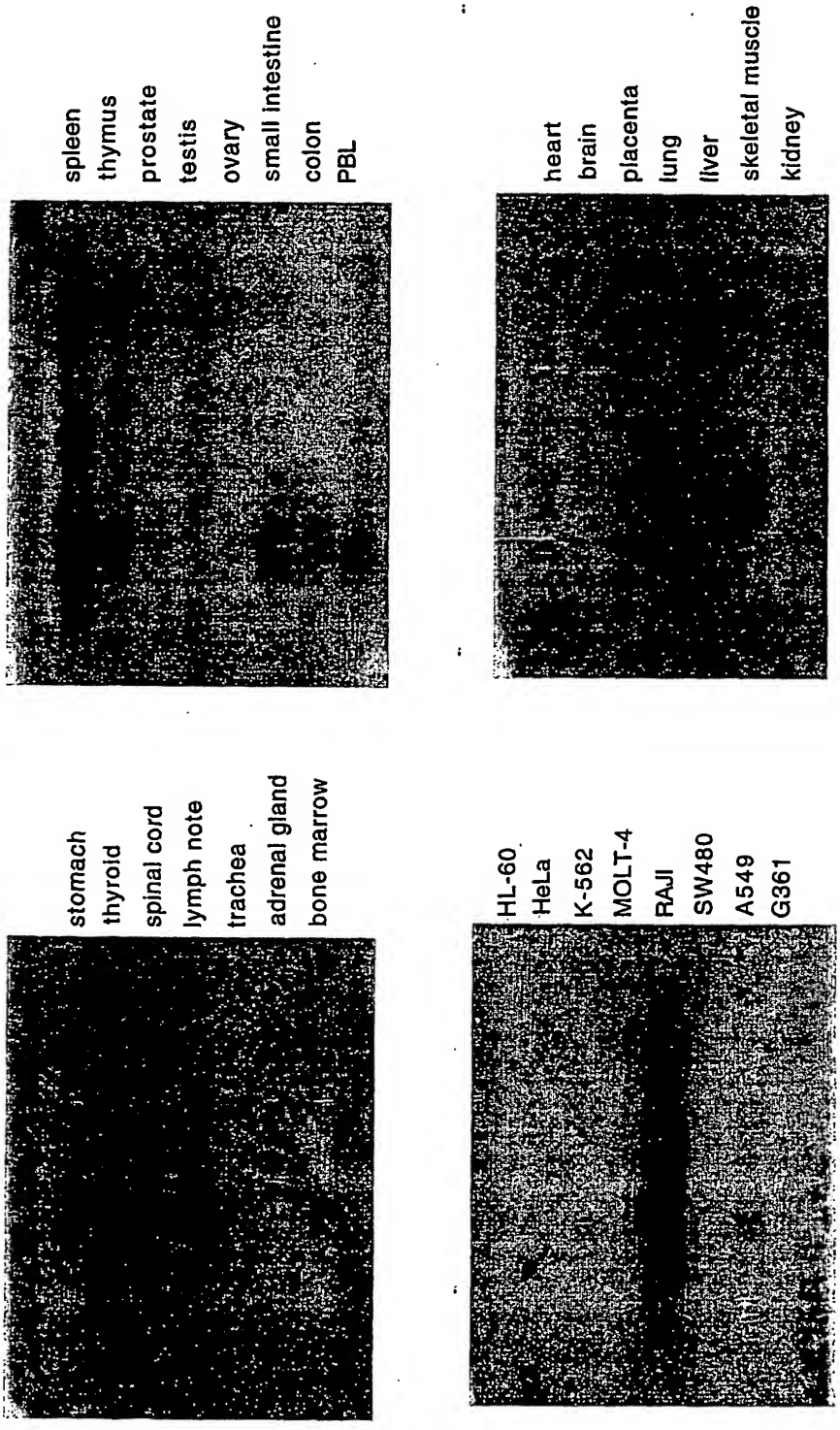
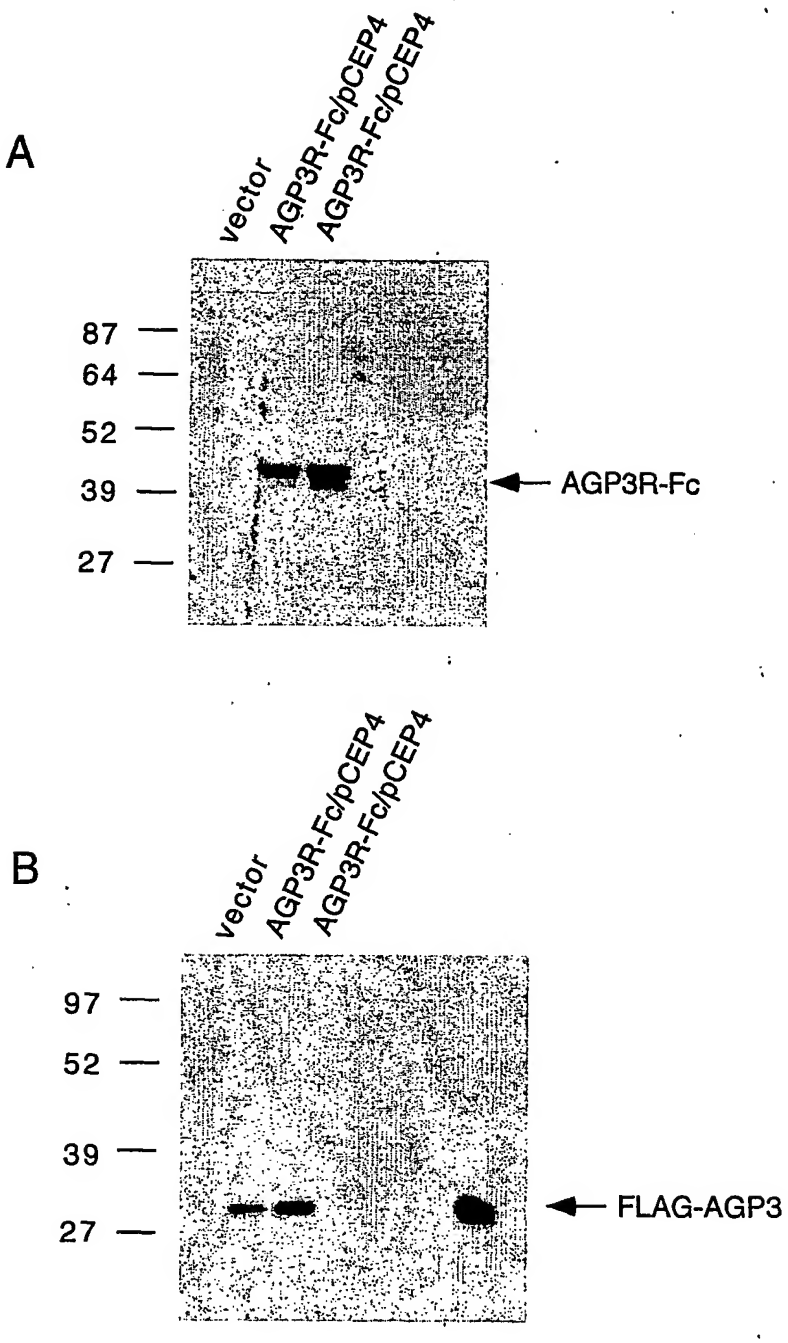


FIG 21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vector
AGP3R-Fc/pCEP4
AGP3R-Fc/pCEP4
87
64
52
39
27
AGP3R-Fc
B
vector
AGP3R-Fc/pCEP4
AGP3R-Fc/pCEP4
97
52
39
27
FLAG-AGP3

SEQUENCE LISTING

<110> AMGEN INC.

<120> RECEPTOR FROM TNF FAMILY

<130> A-570B

<140> NOT YET ASSIGNED

<141> 2001-02-12

<150> 60/181,800

<151> 2000-02-11

<160> 52

<170> PatentIn version 3.0

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acaaacacag ataacaggaa atgatccatt ccctgtggtc acttattcta aaggccccaa 120

ccttcaaagt tcaagtagtg at atg gat gac tcc aca gaa agg gag cag tca 172
Met Asp Asp Ser Thr Glu Arg Glu Gln Ser
1 5 10

cgc ctt act tct tgc ctt aag aaa aga gaa gaa atg aaa ctg aag gag 220
Arg Leu Thr Ser Cys Leu Lys Lys Arg Glu Glu Met Lys Leu Lys Glu

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                15                  20                  25

tgt gtt tcc atc ctc cca cgg aag gaa agc ccc tct gtc cga tcc tcc      268
Cys Val Ser Ile Leu Pro Arg Lys Glu Ser Pro Ser Val Arg Ser Ser
            30                  35                  40

aaa gac gga aag ctg ctg gct gca acc ttg ctg ctg gca ctg ctg tct      316
Lys Asp Gly Lys Leu Leu Ala Ala Thr Leu Leu Leu Ala Leu Leu Ser
        45                  50                  55

tgc tgc ctc acg gtg gtg tct ttc tac cag gtg gcc gcc ctg caa ggg      364
Cys Cys Leu Thr Val Val Ser Phe Tyr Gln Val Ala Ala Leu Gln Gly
    60                  65                  70

gac ctg gcc agc ctc cgg gca gag ctg cag ggc cac cac gcg gag aag      412
Asp Leu Ala Ser Leu Arg Ala Glu Leu Gln Gly His His Ala Glu Lys
75                  80                  85                  90

ctg cca gca gga gca gga gcc ccc aag gcc ggc ctg gag gaa gct cca      460
Leu Pro Ala Gly Ala Gly Ala Pro Lys Ala Gly Leu Glu Glu Ala Pro
                95                  100                 105

gct gtc acc gcg gga ctg aaa atc ttt gaa cca cca gct cca gga gaa      508
Ala Val Thr Ala Gly Leu Lys Ile Phe Glu Pro Pro Ala Pro Gly Glu
            110                 115                 120

ggc aac tcc agt cag aac agc aga aat aag cgt gcc gtt cag ggt cca      556
Gly Asn Ser Ser Gln Asn Ser Arg Asn Lys Arg Ala Val Gln Gly Pro
        125                 130                 135

gaa gaa aca gtc act caa gac tgc ttg caa ctg att gca gac agt gaa      604
Glu Glu Thr Val Thr Gln Asp Cys Leu Gln Leu Ile Ala Asp Ser Glu
    140                 145                 150

aca cca act ata caa aaa gga tct tac aca ttt gtt cca tgg ctt ctc      652
Thr Pro Thr Ile Gln Lys Gly Ser Tyr Thr Phe Val Pro Trp Leu Leu
155                 160                 165                 170

agc ttt aaa agg gga agt gcc cta gaa gaa aaa gag aat aaa ata ttg      700
Ser Phe Lys Arg Gly Ser Ala Leu Glu Glu Lys Glu Asn Lys Ile Leu
                175                 180                 185

gtc aaa gaa act ggt tac ttt ttt ata tat ggt cag gtt tta tat act      748
Val Lys Glu Thr Gly Tyr Phe Phe Ile Tyr Gly Gln Val Leu Tyr Thr
            190                 195                 200

gat aag acc tac gcc atg gga cat cta att cag agg aag aag gtc cat      796
Asp Lys Thr Tyr Ala Met Gly His Leu Ile Gln Arg Lys Lys Val His
        205                 210                 215

gtc ttt ggg gat gaa ttg agt ctg gtg act ttg ttt cga tgt att caa      844
Val Phe Gly Asp Glu Leu Ser Leu Val Thr Leu Phe Arg Cys Ile Gln
    220                 225                 230

aat atg cct gaa aca cta ccc aat aat tcc tgc tat tca gct ggc att      892
Asn Met Pro Glu Thr Leu Pro Asn Asn Ser Cys Tyr Ser Ala Gly Ile
235                 240                 245                 250

gca aaa ctg gaa gaa gga gat gaa ctc caa ctt gca ata cca aga gaa      940
Ala Lys Leu Glu Glu Gly Asp Glu Leu Gln Leu Ala Ile Pro Arg Glu
                255                 260                 265

aat gca caa ata tca ctg gat gga gat gtc aca ttt ttt ggt gca ttg      988
Asn Ala Gln Ile Ser Leu Asp Gly Asp Val Thr Phe Phe Gly Ala Leu
```

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            270                 275                 280

aaa ctg ctg tgacctactt acaccatgtc tgtagctatt ttcctccctt            1037
Lys Leu Leu
        285

tctctgtacc tctaagaaga aagaatctaa ctgaaaatac caaaaaaaaa aaaaaaaaaa  1097

aaaaaaaagt agttaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa  1157

aaaaactcgg aggggg                                                  1173
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            20                  25                  30

Arg Lys Glu Ser Pro Ser Val Arg Ser Ser Lys Asp Gly Lys Leu Leu
        35                  40                  45

Ala Ala Thr Leu Leu Leu Ala Leu Leu Ser Cys Cys Leu Thr Val Val
    50                  55                  60

Ser Phe Tyr Gln Val Ala Ala Leu Gln Gly Asp Leu Ala Ser Leu Arg
65                  70                  75                  80

Ala Glu Leu Gln Gly His His Ala Glu Lys Leu Pro Ala Gly Ala Gly
                85                  90                  95

Ala Pro Lys Ala Gly Leu Glu Glu Ala Pro Ala Val Thr Ala Gly Leu
            100                 105                 110

Lys Ile Phe Glu Pro Pro Ala Pro Gly Glu Gly Asn Ser Ser Gln Asn
        115                 120                 125

Ser Arg Asn Lys Arg Ala Val Gln Gly Pro Glu Glu Thr Val Thr Gln
    130                 135                 140

Asp Cys Leu Gln Leu Ile Ala Asp Ser Glu Thr Pro Thr Ile Gln Lys
145                 150                 155                 160
```

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Gly Ser Tyr Thr Phe Val Pro Trp Leu Leu Ser Phe Lys Arg Gly Ser
                165                 170                 175

Ala Leu Glu Glu Lys Glu Asn Lys Ile Leu Val Lys Glu Thr Gly Tyr
            180                 185                 190

Phe Phe Ile Tyr Gly Gln Val Leu Tyr Thr Asp Lys Thr Tyr Ala Met
        195                 200                 205

Gly His Leu Ile Gln Arg Lys Lys Val His Val Phe Gly Asp Glu Leu
    210                 215                 220

Ser Leu Val Thr Leu Phe Arg Cys Ile Gln Asn Met Pro Glu Thr Leu
225                 230                 235                 240

Pro Asn Asn Ser Cys Tyr Ser Ala Gly Ile Ala Lys Leu Glu Glu Gly
                245                 250                 255

Asp Glu Leu Gln Leu Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser Leu
            260                 265                 270

Asp Gly Asp Val Thr Phe Phe Gly Ala Leu Lys Leu Leu
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<222> (52)..(978)

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Glu Ser Ala Lys Thr Leu Pro Pro Pro Cys Leu Cys Phe Cys Ser Glu
        5                   10                  15

aaa gga gaa gat atg aaa gtg gga tat gat ccc atc act ccg cag aag      153
Lys Gly Glu Asp Met Lys Val Gly Tyr Asp Pro Ile Thr Pro Gln Lys
    20                  25                  30
```

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gag gag ggt gcc tgg ttt ggg atc tgc agg gat gga agg ctg ctg gct      201
Glu Glu Gly Ala Trp Phe Gly Ile Cys Arg Asp Gly Arg Leu Leu Ala
35                  40                  45                  50

gct acc ctc ctg ctg gcc ctg ttg tcc agc agt ttc aca gcg atg tcc      249
Ala Thr Leu Leu Leu Ala Leu Leu Ser Ser Ser Phe Thr Ala Met Ser
                55                  60                  65

ttg tac cag ttg gct gcc ttg caa gca gac ctg atg aac ctg cgc atg      297
Leu Tyr Gln Leu Ala Ala Leu Gln Ala Asp Leu Met Asn Leu Arg Met
            70                  75                  80

gag ctg cag agc tac cga ggt tca gca aca cca gcc gcc gcg ggt gct      345
Glu Leu Gln Ser Tyr Arg Gly Ser Ala Thr Pro Ala Ala Ala Gly Ala
        85                  90                  95

cca gag ttg acc gct gga gtc aaa ctc ctg aca ccg gca gct cct cga      393
Pro Glu Leu Thr Ala Gly Val Lys Leu Leu Thr Pro Ala Ala Pro Arg
    100                 105                 110

ccc cac aac tcc agc cgc ggc cac agg aac aga cgc gct ttc cag gga      441
Pro His Asn Ser Ser Arg Gly His Arg Asn Arg Arg Ala Phe Gln Gly
115                 120                 125                 130

cca gag gaa aca gaa caa gat gta gac ctc tca gct cct cct gca cca      489
Pro Glu Glu Thr Glu Gln Asp Val Asp Leu Ser Ala Pro Pro Ala Pro
                135                 140                 145

tgc ctg cct gga tgc cgc cat tct caa cat gat gat aat gga atg aac      537
Cys Leu Pro Gly Cys Arg His Ser Gln His Asp Asp Asn Gly Met Asn
            150                 155                 160

ctc aga aac atc att caa gac tgt ctg cag ctg att gca gac agc gac      585
Leu Arg Asn Ile Ile Gln Asp Cys Leu Gln Leu Ile Ala Asp Ser Asp
        165                 170                 175

acg ccg act ata cga aaa gga act tac aca ttt gtt cca tgg ctt ctc      633
Thr Pro Thr Ile Arg Lys Gly Thr Tyr Thr Phe Val Pro Trp Leu Leu
    180                 185                 190

agc ttt aaa aga gga aat gcc ttg gag gag aaa gag aac aaa ata gtg      681
Ser Phe Lys Arg Gly Asn Ala Leu Glu Glu Lys Glu Asn Lys Ile Val
195                 200                 205                 210

gtg agg caa aca ggc tat ttc ttc atc tac agc cag gtt cta tac acg      729
Val Arg Gln Thr Gly Tyr Phe Phe Ile Tyr Ser Gln Val Leu Tyr Thr
                215                 220                 225

gac ccc atc ttt gct atg ggt cat gtc atc cag agg aag aaa gta cac      777
Asp Pro Ile Phe Ala Met Gly His Val Ile Gln Arg Lys Lys Val His
            230                 235                 240

gtc ttt ggg gac gag ctg agc ctg gtg acc ctg ttc cga tgt att cag      825
Val Phe Gly Asp Glu Leu Ser Leu Val Thr Leu Phe Arg Cys Ile Gln
        245                 250                 255

aat atg ccc aaa aca ctg ccc aac aat tcc tgc tac ttg gct ggc atc      873
Asn Met Pro Lys Thr Leu Pro Asn Asn Ser Cys Tyr Leu Ala Gly Ile
    260                 265                 270

gcg agg ctg gaa gaa gga gat gag att cag ctt gca att cct cgg gag      921
Ala Arg Leu Glu Glu Gly Asp Glu Ile Gln Leu Ala Ile Pro Arg Glu
275                 280                 285                 290
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aat gca cag att tca cgc aac gga gac gac acc ttc ttt ggt gcc cta 969
Asn Ala Gln Ile Ser Arg Asn Gly Asp Asp Thr Phe Phe Gly Ala Leu
295 300 305

aaa ctg ctg taactcactt gctggagtgc gtgatcccct tccctcgtct 1018
Lys Leu Leu

tctctgtacc tccgagggag aaacagacga ctggaaaaat aaaagatggg gaaagccgtc 1078

agcgaaagtt ttctcgtgac ccgttgaatc tgatccaaac caggaaatat aacagacagc 1138

c 1139

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20 25 30

Gln Lys Glu Glu Gly Ala Trp Phe Gly Ile Cys Arg Asp Gly Arg Leu
35 40 45

Leu Ala Ala Thr Leu Leu Leu Ala Leu Leu Ser Ser Ser Phe Thr Ala
50 55 60

Met Ser Leu Tyr Gln Leu Ala Ala Leu Gln Ala Asp Leu Met Asn Leu
65 70 75 80

Arg Met Glu Leu Gln Ser Tyr Arg Gly Ser Ala Thr Pro Ala Ala Ala
85 90 95

Gly Ala Pro Glu Leu Thr Ala Gly Val Lys Leu Leu Thr Pro Ala Ala
100 105 110

Pro Arg Pro His Asn Ser Ser Arg Gly His Arg Asn Arg Arg Ala Phe
115 120 125

Gln Gly Pro Glu Glu Thr Glu Gln Asp Val Asp Leu Ser Ala Pro Pro
130 135 140

Ala Pro Cys Leu Pro Gly Cys Arg His Ser Gln His Asp Asp Asn Gly

145 150 155 160

Met Asn Leu Arg Asn Ile Ile Gln Asp Cys Leu Gln Leu Ile Ala Asp
165 170 175

Ser Asp Thr Pro Thr Ile Arg Lys Gly Thr Tyr Thr Phe Val Pro Trp
180 185 190

Leu Leu Ser Phe Lys Arg Gly Asn Ala Leu Glu Glu Lys Glu Asn Lys
195 200 205

Ile Val Val Arg Gln Thr Gly Tyr Phe Phe Ile Tyr Ser Gln Val Leu
210 215 220

Tyr Thr Asp Pro Ile Phe Ala Met Gly His Val Ile Gln Arg Lys Lys
225 230 235 240

Val His Val Phe Gly Asp Glu Leu Ser Leu Val Thr Leu Phe Arg Cys
245 250 255

Ile Gln Asn Met Pro Lys Thr Leu Pro Asn Asn Ser Cys Tyr Leu Ala
260 265 270

Gly Ile Ala Arg Leu Glu Glu Gly Asp Glu Ile Gln Leu Ala Ile Pro
275 280 285

Arg Glu Asn Ala Gln Ile Ser Arg Asn Gly Asp Asp Thr Phe Phe Gly
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Ala Leu Lys Leu Leu
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<212> PRT

<213> Homo sapiens

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1 5 10 15

Xaa Xaa Lys Xaa Glu Xaa Met Lys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
20 25 30

Xaa Xaa Xaa Glu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asp Gly Xaa Leu
35 40 45

Leu Ala Ala Thr Leu Leu Leu Ala Leu Leu Ser Xaa Xaa Xaa Thr Xaa
50 55 60

Xaa Ser Xaa Tyr Gln Xaa Ala Ala Leu Gln Xaa Asp Leu Xaa Xaa Leu
65 70 75 80

Arg Xaa Glu Leu Gln Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Pro Ala Xaa Ala
85 90 95

Gly Ala Pro Xaa Xaa Thr Ala Gly Xaa Lys Xaa Xaa Xaa Pro Xaa Ala
100 105 110

Pro Xaa Xaa Xaa Asn Ser Ser Xaa Xaa Xaa Arg Asn Xaa Arg Ala Xaa
115 120 125

Gln Gly Pro Glu Glu Thr Xaa Xaa Gln Asp Cys Leu Gln Leu Ile Ala
130 135 140

Asp Ser Xaa Thr Pro Thr Ile Xaa Lys Gly Xaa Tyr Thr Phe Val Pro
145 150 155 160

Trp Leu Leu Ser Phe Lys Arg Gly Ser Ala Leu Glu Glu Lys Glu Asn
165 170 175

Lys Ile Xaa Val Xaa Xaa Thr Gly Tyr Phe Phe Ile Tyr Xaa Gln Val
180 185 190

Leu Tyr Thr Asp Xaa Xaa Xaa Ala Met Gly His Xaa Ile Gln Arg Lys
195 200 205

Lys Val His Val Phe Gly Asp Glu Leu Ser Leu Val Thr Leu Phe Arg
210 215 220

Cys Ile Gln Asn Met Pro Xaa Thr Leu Pro Asn Asn Ser Cys Tyr Ser
225 230 235 240

Ala Gly Ile Ala Xaa Leu Glu Glu Gly Asp Glu Xaa Gln Leu Ala Ile
245 250 255

Pro Arg Glu Asn Ala Gln Ile Ser Xaa Xaa Gly Asp Xaa Thr Phe Phe
260 265 270

Gly Ala Leu Lys Leu Leu
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<213> Consensus

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Ser Xaa Gly Val Xaa Leu Xaa Asn Xaa Leu Val Val Xaa Gly Leu Tyr
20 25 30

Phe Ile Tyr Ser Gln Val Xaa Phe Xaa Gly Gln Xaa Cys Pro Xaa Val
35 40 45

Xaa Leu Xaa His Xaa Val Xaa Val Xaa Tyr Pro Xaa Leu Leu Ser Xaa
50 55 60

Thr Xaa Cys Xaa Trp Xaa Ser Xaa Tyr Leu Gly Gly Val Phe Xaa Leu
65 70 75 80

Xaa Gly Asp Xaa Leu Tyr Val Asn Val Xaa Ser Xaa Phe Xaa Thr Phe
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Phe Gly Leu Phe Lys Leu
100

<210> 7

<211> 143

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<213> Homo sapiens

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20 25 30

Leu Ser Gly Val Lys Tyr Lys Lys Gly Gly Leu Val Leu Asn Glu Thr
35 40 45

Gly Leu Tyr Phe Val Tyr Ser Lys Val Tyr Phe Arg Gly Gln Ser Cys
50 55 60

Asn Asn Leu Pro Leu Ser His Lys Val Tyr Met Arg Asn Ser Lys Tyr
65 70 75 80

Pro Gln Asp Leu Val Met Met Glu Gly Lys Met Met Ser Tyr Cys Thr
85 90 95

Thr Gly Gln Met Trp Ala Arg Ser Ser Tyr Leu Gly Ala Val Phe Asn
100 105 110

Leu Thr Ser Ala Asp His Leu Tyr Val Asn Val Ser Glu Leu Ser Leu
115 120 125

Val Asn Phe Glu Glu Ser Gln Thr Phe Phe Gly Leu Tyr Lys Leu
130 135 140

<210> 8

<211> 143

<212> PRT

<213> Mus musculus

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Glu Lys Lys Glu Pro Arg Ser Val Ala His Leu Thr Gly Asn Pro His
1 5 10 15

Ser Arg Ser Ile Pro Leu Glu Trp Glu Asp Thr Tyr Gly Thr Ala Leu
20 25 30

Ile Ser Gly Val Lys Tyr Lys Lys Gly Gly Leu Val Ile Asn Glu Thr
35 40 45

Gly Leu Tyr Phe Val Tyr Ser Lys Val Tyr Phe Arg Gly Gln Ser Cys
50 55 60

Asn Asn Gln Pro Ile Asn His Lys Val Tyr Met Arg Asn Ser Lys Tyr
65 70 75 80

Pro Glu Asp Leu Val Leu Met Glu Glu Lys Arg Leu Asn Tyr Cys Thr
85 90 95

Thr Gly Gln Ile Trp Ala His Ser Ser Tyr Leu Gly Ala Val Phe Asn
100 105 110

Leu Thr Ser Ala Asp His Leu Val Tyr Asn Ile Ser Gln Leu Ser Leu
115 120 125

Ile Asn Phe Glu Glu Ser Lys Thr Phe Phe Gly Leu Tyr Lys Leu
130 135 140

<210> 9

<211> 143

<212> PRT

<213> Rattus rattus

<400> 9

Glu Thr Lys Lys Pro Arg Ser Val Ala His Leu Thr Gly Asn Pro Arg
1 5 10 15

Ser Arg Ser Ile Pro Leu Glu Trp Glu Asp Thr Tyr Gly Thr Ala Leu
20 25 30

Ile Ser Gly Val Lys Tyr Lys Lys Gly Gly Leu Val Ile Asn Glu Ala

35 40 45

Gly Leu Tyr Phe Val Tyr Ser Lys Val Tyr Phe Arg Gly Gln Ser Cys
50 55 60

Asn Ser Gln Pro Leu Ser His Lys Val Tyr Met Arg Asn Phe Lys Tyr
65 70 75 80

Pro Gly Asp Leu Val Leu Met Glu Glu Lys Lys Leu Asn Tyr Cys Thr
85 90 95

Thr Gly Gln Ile Trp Ala His Ser Ser Tyr Leu Gly Ala Val Phe Asn
100 105 110

Leu Thr Val Ala Asp His Leu Tyr Val Asn Ile Ser Gln Leu Ser Leu
115 120 125

Ile Asn Phe Glu Glu Ser Lys Thr Phe Phe Gly Leu Tyr Lys Leu
130 135 140

<210> 10

<211> 146

<212> PRT

<213> Homo sapiens

<400> 10

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1 5 10 15

Ser Lys Thr Thr Ser Val Leu Gln Trp Ala Glu Lys Gly Tyr Tyr Thr
20 25 30

Met Ser Asn Asn Leu Val Thr Leu Glu Asn Gly Lys Gln Leu Thr Val
35 40 45

Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr Ala Gln Val Thr Phe Cys Ser
50 55 60

Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe Ile Ala Ser Leu Cys Leu
65 70 75 80

Lys Ser Pro Gly Arg Phe Glu Arg Ile Leu Leu Arg Ala Ala Asn Thr
85 90 95

His Ser Ser Ala Lys Pro Cys Gly Gln Gln Ser Ile His Leu Gly Gly
100 105 110

Val Phe Glu Leu Gln Pro Gly Ala Ser Val Phe Val Asn Val Thr Asp
115 120 125

Pro Ser Gln Val Ser His Gly Thr Gly Phe Thr Ser Phe Gly Leu Leu
130 135 140

Lys Leu
145

<210> 11

<211> 146

<212> PRT

<213> Mus musculus

<400> 11

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1               5                  10                  15

Ser Asn Ala Ala Ser Val Leu Gln Trp Ala Lys Lys Gly Tyr Tyr Thr
            20                  25                  30

Met Lys Ser Asn Leu Val Met Leu Glu Asn Gly Lys Gln Leu Thr Val
        35                  40                  45

Lys Arg Glu Gly Leu Tyr Tyr Val Tyr Thr Gln Val Thr Phe Gln Ser
    50                  55                  60

Asn Arg Glu Pro Ser Ser Gln Arg Pro Phe Ile Val Gly Leu Trp Leu
65                  70                  75                  80

Lys Pro Ser Ile Gly Ser Glu Arg Ile Leu Leu Lys Ala Ala Asn Thr
                85                  90                  95

His Ser Ser Ser Gln Leu Cys Glu Gln Gln Ser Val His Leu Gly Gly
            100                 105                 110

Val Phe Glu Leu Gln Ala Gly Ala Ser Val Phe Val Asn Val Thr Glu
        115                 120                 125

Ala Ser Gln Val Ile His Arg Val Gly Phe Ser Ser Phe Gly Leu Leu
    130                 135                 140

Lys Leu
145
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<210> 12

<211> 144

<212> PRT

<213> Homo sapiens

<400> 12

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Val Thr Gln Asp Cys Leu Gln Leu Ile Ala Asp Ser Glu Thr Pro Thr
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Ile Gln Lys Gly Ser Tyr Thr Phe Val Pro Trp Leu Leu Ser Phe Lys
            20                  25                  30

Arg Gly Ser Ala Leu Glu Glu Lys Glu Asn Lys Ile Leu Val Lys Glu
        35                  40                  45

Thr Gly Tyr Phe Phe Ile Tyr Gly Gln Val Leu Tyr Thr Asp Lys Thr
    50                  55                  60
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Tyr Ala Met Gly His Leu Ile Gln Arg Lys Lys Val His Val Phe Gly
65 70 75 80

Asp Glu Leu Ser Leu Val Thr Leu Phe Arg Cys Ile Gln Asn Met Pro
85 90 95

Glu Thr Leu Pro Asn Asn Ser Cys Tyr Ser Ala Gly Ile Ala Lys Leu
100 105 110

Glu Glu Gly Asp Glu Leu Gln Leu Ala Ile Pro Arg Glu Asn Ala Gln
115 120 125

Ile Ser Leu Asp Gly Asp Val Thr Phe Phe Gly Ala Leu Lys Leu Leu
130 135 140

<210> 13

<211> 147

<212> PRT

<213> Mus musculus

<400> 13

Leu Arg Asn Ile Ile Gln Asp Cys Leu Gln Leu Ile Ala Asp Ser Asp
1 5 10 15

Thr Pro Thr Ile Arg Lys Gly Thr Tyr Thr Phe Val Pro Trp Leu Leu
20 25 30

Ser Phe Lys Arg Gly Asn Ala Leu Glu Glu Lys Glu Asn Lys Ile Val
35 40 45

Val Arg Gln Thr Gly Tyr Phe Phe Ile Tyr Ser Gln Val Leu Tyr Thr
50 55 60

Asp Pro Ile Phe Ala Met Gly His Val Ile Gln Arg Lys Lys Val His
65 70 75 80

Val Phe Gly Asp Glu Leu Ser Leu Val Thr Leu Phe Arg Cys Ile Gln
85 90 95

Asn Met Pro Lys Thr Leu Pro Asn Asn Ser Cys Tyr Ser Ala Gly Ile
100 105 110

Ala Arg Leu Glu Glu Gly Asp Glu Ile Gln Leu Ala Ile Pro Arg Glu
115 120 125

Asn Ala Gln Ile Ser Arg Asn Gly Asp Asp Thr Phe Phe Gly Ala Leu
130 135 140

Lys Leu Leu
145

<210> 14

<211> 160

<212> PRT

<213> Mus musculus

<400> 14

Gly Lys Pro Glu Ala Gln Pro Phe Ala His Leu Thr Ile Asn Ala Ala
1 5 10 15

Ser Ile Pro Ser Gly Ser His Lys Val Thr Leu Ser Ser Trp Tyr His
20 25 30

Asp Arg Gly Trp Ala Lys Ile Ser Asn Met Thr Leu Ser Asn Gly Lys
35 40 45

Leu Arg Val Asn Gln Asp Gly Phe Tyr Tyr Leu Tyr Ala Asn Ile Cys
50 55 60

Phe Arg His His Glu Thr Ser Gly Ser Val Pro Thr Asp Tyr Leu Gln
65 70 75 80

Leu Met Val Tyr Val Val Lys Thr Ser Ile Lys Ile Pro Ser Ser His
85 90 95

Asn Leu Met Lys Gly Gly Ser Thr Lys Asn Trp Ser Gly Asn Ser Glu
100 105 110

Phe His Phe Tyr Ser Ile Asn Val Gly Gly Phe Phe Lys Leu Arg Ala
115 120 125

Gly Glu Glu Ile Ser Ile Gln Val Ser Asn Pro Ser Leu Leu Asp Pro
130 135 140

Asp Gln Asp Ala Thr Tyr Phe Gly Ala Phe Lys Val Gln Asp Ile Asp
145 150 155 160

<210> 15

<211> 160

<212> PRT

<213> Homo sapiens

<400> 15

Ser Lys Leu Glu Ala Gln Pro Phe Ala His Leu Thr Ile Asn Ala Thr
1 5 10 15

Asp Ile Pro Ser Gly Ser His Lys Val Ser Leu Ser Ser Trp Tyr His
20 25 30

Asp Arg Gly Trp Ala Lys Ile Ser Asn Met Thr Phe Ser Asn Gly Lys
35 40 45

Leu Ile Val Asn Gln Asp Gly Phe Tyr Tyr Leu Tyr Ala Asn Ile Cys
50 55 60

Phe Arg His His Glu Thr Ser Gly Asp Leu Ala Thr Glu Tyr Leu Gln
65 70 75 80

Leu Met Val Tyr Val Thr Lys Thr Ser Ile Lys Ile Pro Ser Ser His

85 90 95

Thr Leu Met Lys Gly Gly Ser Thr Lys Tyr Trp Ser Gly Asn Ser Glu
100 105 110

Phe His Phe Tyr Ser Ile Asn Val Gly Gly Phe Phe Lys Leu Arg Ser
115 120 125

Gly Glu Glu Ile Ser Ile Glu Val Ser Asn Pro Ser Leu Leu Asp Pro
130 135 140

Asp Gln Asp Ala Thr Tyr Phe Gly Ala Phe Lys Val Arg Asp Ile Asp
145 150 155 160

<210> 16

<211> 166

<212> PRT

<213> Homo sapiens

<400> 16

Glu Arg Gly Pro Gln Arg Val Ala Ala His Ile Thr Gly Thr Arg Gly
1 5 10 15

Arg Ser Asn Thr Leu Ser Ser Pro Asn Ser Lys Asn Glu Lys Ala Leu
20 25 30

Gly Arg Lys Ile Asn Ser Trp Glu Ser Ser Arg Ser Gly His Ser Phe
35 40 45

Leu Ser Asn Leu His Leu Arg Asn Gly Glu Leu Val Ile His Glu Lys
50 55 60

Gly Phe Tyr Tyr Ile Tyr Ser Gln Thr Tyr Phe Arg Phe Gln Glu Glu
65 70 75 80

Ile Lys Glu Asn Thr Lys Asn Asp Lys Gln Met Val Gln Tyr Ile Tyr
85 90 95

Lys Tyr Thr Ser Tyr Pro Asp Pro Ile Leu Leu Met Lys Ser Ala Arg
100 105 110

Asn Ser Cys Trp Ser Lys Asp Ala Glu Tyr Gly Leu Tyr Ser Ile Tyr
115 120 125

Gln Gly Gly Ile Phe Glu Leu Lys Glu Asn Asp Arg Ile Phe Val Ser
130 135 140

Val Thr Asn Glu His Leu Ile Asp Met Asp His Glu Ala Ser Phe Phe
145 150 155 160

Gly Ala Phe Leu Val Gly
165

<210> 17

<211> 172

<212> PRT

<213> Mus musculus

<400> 17

Gly Gly Arg Pro Gln Lys Val Ala Ala His Ile Thr Gly Ile Thr Arg
1 5 10 15

Arg Ser Asn Ser Ala Leu Ile Pro Ile Ser Lys Asp Gly Lys Thr Leu
20 25 30

Gly Gln Lys Ile Glu Ser Trp Glu Ser Ser Arg Lys Gly His Ser Phe
35 40 45

Leu Asn His Val Leu Phe Arg Asn Gly Glu Leu Val Ile Glu Gln Glu
50 55 60

Gly Leu Tyr Tyr Ile Tyr Ser Gln Thr Tyr Phe Arg Phe Gln Glu Ala
65 70 75 80

Glu Asp Ala Ser Lys Met Val Ser Lys Asp Lys Val Arg Thr Lys Gln
85 90 95

Leu Val Gln Tyr Ile Tyr Lys Tyr Thr Ser Tyr Pro Asp Pro Ile Val
100 105 110

Leu Met Lys Ser Ala Arg Asn Ser Cys Trp Ser Arg Asp Ala Glu Tyr
115 120 125

Gly Leu Tyr Ser Ile Tyr Gln Gly Gly Leu Phe Glu Leu Lys Lys Asn
130 135 140

Asp Arg Ile Phe Val Ser Val Thr Asn Glu His Leu Met Asp Leu Asp
145 150 155 160

Gln Glu Ala Ser Phe Phe Gly Ala Phe Leu Ile Asn
165 170

<210> 18

<211> 143

<212> PRT

<213> Homo sapiens

<400> 18

Arg Ala Pro Phe Lys Lys Ser Trp Ala Tyr Leu Gln Val Ala Lys His
1 5 10 15

Leu Asn Lys Thr Lys Leu Ser Trp Asn Lys Asp Gly Ile Leu His Gly
20 25 30

Val Arg Tyr Gln Asp Gly Asn Leu Val Ile Gln Phe Pro Gly Leu Tyr
35 40 45

Phe Ile Ile Cys Gln Leu Gln Phe Leu Val Gln Cys Pro Asn Asn Ser
50 55 60

Val Asp Leu Lys Leu Glu Leu Leu Ile Asn Lys His Ile Lys Lys Gln
65 70 75 80

Ala Leu Val Thr Val Cys Glu Ser Gly Met Gln Thr Lys His Val Tyr
85 90 95

Gln Asn Leu Ser Gln Phe Leu Leu Asp Tyr Leu Gln Val Asn Thr Thr
100 105 110

Ile Ser Val Asn Val Asp Thr Phe Gln Tyr Ile Asp Thr Ser Thr Phe
115 120 125

Pro Leu Glu Asn Val Leu Ser Ile Phe Leu Tyr Ser Asn Ser Asp
130 135 140

<210> 19

<211> 143

<212> PRT

<213> Mus musculus

<400> 19

Ser Thr Pro Ser Lys Lys Ser Trp Ala Tyr Leu Gln Val Ser Lys His
1 5 10 15

Leu Asn Asn Thr Lys Leu Ser Trp Asn Glu Asp Gly Thr Ile His Gly
20 25 30

Leu Ile Tyr Gln Asp Gly Asn Leu Ile Val Gln Phe Pro Gly Leu Tyr
35 40 45

Phe Ile Val Cys Gln Leu Gln Phe Leu Val Gln Cys Ser Asn His Ser
50 55 60

Val Asp Leu Thr Leu Gln Leu Leu Ile Asn Ser Lys Ile Lys Lys Gln
65 70 75 80

Thr Leu Val Thr Val Cys Glu Ser Gly Val Gln Ser Lys Asn Ile Tyr
85 90 95

Gln Asn Leu Ser Gln Phe Leu Leu His Tyr Leu Gln Val Asn Ser Thr
100 105 110

Ile Ser Val Arg Val Asp Asn Phe Gln Tyr Val Asp Thr Asn Thr Phe
115 120 125

Pro Leu Asp Asn Val Leu Ser Val Phe Leu Tyr Ser Ser Ser Asp
130 135 140

<210> 20

<211> 163

<212> PRT

<213> Homo sapiens

<400> 20

Asp Leu Ser Pro Gly Leu Pro Ala Ala His Leu Ile Gly Ala Pro Leu
1 5 10 15

Lys Gly Gln Gly Leu Gly Trp Glu Thr Thr Lys Glu Gln Ala Phe Leu
20 25 30

Thr Ser Gly Thr Gln Phe Ser Asp Ala Glu Gly Leu Ala Leu Pro Gln
35 40 45

Asp Gly Leu Tyr Tyr Leu Tyr Cys Leu Val Gly Tyr Arg Gly Arg Ala
50 55 60

Pro Pro Gly Gly Gly Asp Pro Gln Gly Arg Ser Val Thr Leu Arg Ser
65 70 75 80

Ser Leu Tyr Arg Ala Gly Gly Ala Tyr Gly Pro Gly Thr Pro Glu Leu
85 90 95

Leu Leu Glu Gly Ala Glu Thr Val Thr Pro Val Leu Asp Pro Ala Arg
100 105 110

Arg Gln Gly Tyr Gly Pro Leu Trp Tyr Thr Ser Val Gly Phe Gly Gly
115 120 125

Leu Val Gln Leu Arg Arg Gly Glu Arg Val Tyr Val Asn Ile Ser His
130 135 140

Pro Asp Met Val Asp Phe Ala Arg Gly Lys Thr Phe Phe Gly Ala Val
145 150 155 160

Met Val Gly

<210> 21

<211> 159

<212> PRT

<213> Mus musculus

<400> 21

Asp Leu Asn Pro Glu Leu Pro Ala Ala His Leu Ile Gly Ala Trp Met
1 5 10 15

Ser Gly Gln Gly Leu Ser Trp Glu Ala Ser Gln Glu Glu Ala Phe Leu
20 25 30

Arg Ser Gly Ala Gln Phe Ser Pro Thr His Gly Leu Ala Leu Pro Gln
35 40 45

Asp Gly Val Tyr Tyr Leu Tyr Cys His Val Gly Tyr Arg Gly Arg Thr
50 55 60

Pro Pro Ala Gly Arg Ser Arg Ala Arg Ser Leu Thr Leu Arg Ser Ala
65 70 75 80

Leu Tyr Arg Ala Gly Gly Ala Tyr Gly Arg Gly Ser Pro Glu Leu Leu
85 90 95

Leu Glu Gly Ala Glu Thr Val Thr Pro Val Val Asp Pro Ile Gly Tyr
100 105 110

Gly Ser Leu Trp Tyr Thr Ser Val Gly Phe Gly Gly Leu Ala Gln Leu
115 120 125

Arg Ser Gly Glu Arg Val Tyr Val Asn Ile Ser His Pro Asp Met Val
130 135 140

Asp Tyr Arg Arg Gly Lys Thr Phe Phe Gly Ala Val Met Val Gly
145 150 155

<210> 22

<211> 149

<212> PRT

<213> Homo sapiens

<400> 22

Ala His Ser Thr Leu Lys Pro Ala Ala His Leu Ile Gly Asp Pro Ser
1 5 10 15

Lys Gln Asn Ser Leu Leu Trp Arg Ala Asn Thr Asp Arg Ala Phe Leu
20 25 30

Gln Asp Gly Phe Ser Leu Ser Asn Asn Ser Leu Leu Val Pro Thr Ser
35 40 45

Gly Ile Tyr Phe Val Tyr Ser Gln Val Val Phe Ser Gly Lys Ala Tyr
50 55 60

Ser Pro Lys Ala Thr Ser Ser Pro Leu Tyr Leu Ala His Glu Val Gln
65 70 75 80

Leu Phe Ser Ser Gln Tyr Pro Phe His Val Pro Leu Leu Ser Ser Gln
85 90 95

Lys Met Val Tyr Pro Gly Leu Gln Glu Pro Trp Leu His Ser Met Tyr
100 105 110

His Gly Ala Ala Phe Gln Leu Thr Gln Gly Asp Gln Leu Ser Thr His
115 120 125

Thr Asp Gly Ile Pro His Leu Val Leu Ser Pro Ser Thr Val Phe Phe
130 135 140

Gly Ala Phe Ala Leu
145

<210> 23

<211> 149

<212> PRT

<213> Mus musculus

<400> 23

Thr His Gly Ile Leu Lys Pro Ala Ala His Leu Val Gly Tyr Pro Ser
1 5 10 15

Lys Gln Asn Ser Leu Leu Trp Arg Ala Ser Thr Asp Arg Ala Phe Leu
20 25 30

Arg His Gly Phe Ser Leu Ser Asn Asn Ser Leu Leu Ile Pro Thr Ser
35 40 45

Gly Leu Tyr Phe Val Tyr Ser Gln Val Val Phe Ser Gly Glu Ser Cys
50 55 60

Ser Pro Arg Ala Ile Pro Thr Pro Ile Tyr Leu Ala His Glu Val Gln
65 70 75 80

Leu Phe Ser Ser Gln Tyr Pro Phe His Val Pro Leu Leu Ser Ala Gln
85 90 95

Lys Ser Val Tyr Pro Gly Leu Gln Gly Pro Trp Val Arg Ser Met Tyr
100 105 110

Gln Gly Ala Val Phe Leu Leu Ser Lys Gly Asp Gln Leu Ser Thr His
115 120 125

Thr Asp Gly Ile Ser His Leu His Phe Ser Pro Ser Ser Val Phe Phe
130 135 140

Gly Ala Phe Ala Leu
145

<210> 24

<211> 152

<212> PRT

<213> Homo sapiens

<400> 24

Arg Thr Pro Ser Asp Lys Pro Val Ala His Val Val Ala Asn Pro Gln
1 5 10 15

Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn Ala Leu Leu
20 25 30

Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val Val Pro Ser Glu
35 40 45

Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly Gln Gly Cys
50 55 60

Pro Ser Thr His Val Leu Leu Thr His Thr Ile Ser Arg Ile Ala Val
65 70 75 80

Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala Ile Lys Ser Pro Cys

85 90 95

Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu Pro
100 105 110

Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly Asp Arg Leu Ser
115 120 125

Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe Ala Glu Ser Gly Gln
130 135 140

Val Tyr Phe Gly Ile Ile Ala Leu
145 150

<210> 25

<211> 29

<212> PRT

<213> Artificial

<220>

<223> Description of Artificial Sequence:AGP-3 RELATED PROTEIN

<220>

<221> misc_feature

<223> Positions 11, 16, 19, X = any naturally occurring amino acid residue

<400> 25

Gln Asp Cys Leu Gln Leu Ile Ala Asp Ser Xaa Thr Pro Thr Ile Xaa
1 5 10 15

Lys Gly Xaa Tyr Thr Phe Val Pro Trp Leu Leu Ser Phe
20 25

<210> 26

<211> 25

<212> PRT

<213> Artificial

<220>

<223> Description of Artificial Sequence:CONSENSUS

<220>

<221> misc_feature

<223> Position 5, X = any naturally occurring amino acid residue.

<400> 26

Ala Met Gly His Xaa Ile Gln Arg Lys Lys Val His Val Phe Gly Asp
1 5 10 15

Glu Leu Ser Leu Val Thr Leu Phe Arg
20 25

<210> 27

<211> 142

<212> PRT

<213> Artificial

<220>

<223> Description of Artificial Sequence:CONSENSUS

<220>

<221> misc_feature

<223> Positions 43, 45, 46, 54, 61-63, 68, 95, 109, 116, 129, 130, 133:
X = any naturally occurring amino acid residue

<400> 27

Gln Asp Cys Leu Gln Leu Ile Ala Asp Ser Xaa Thr Pro Thr Ile Xaa
1 5 10 15

Lys Gly Xaa Tyr Thr Phe Val Pro Trp Leu Leu Ser Phe Lys Arg Gly
20 25 30

Xaa Ala Leu Glu Glu Lys Glu Asn Lys Ile Xaa Val Xaa Xaa Thr Gly
35 40 45

Tyr Phe Phe Ile Tyr Xaa Gln Val Leu Tyr Thr Asp Xaa Xaa Xaa Ala
50 55 60

Met Gly His Xaa Ile Gln Arg Lys Lys Val His Val Phe Gly Asp Glu
65 70 75 80

Leu Ser Leu Val Thr Leu Phe Arg Cys Ile Gln Asn Met Pro Xaa Thr
85 90 95

Leu Pro Asn Asn Ser Cys Tyr Ser Ala Gly Ile Ala Xaa Leu Glu Glu
100 105 110

Gly Asp Glu Xaa Gln Leu Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser
115 120 125

Xaa Xaa Gly Asp Xaa Thr Phe Phe Gly Ala Leu Lys Leu Leu
130 135 140

<210> 28

<211> 20

<212> DNA

<213> Mus musculus

<400> 28
aattaaccct cactaaaggg 20

<210> 29

<211> 33

<212> DNA

<213> Mus musculus

<400> 29
tctccctcga gatcacgcac tccagcaagt gag 33

<210> 30

<211> 24

<212> DNA

<213> Mus musculus

<400> 30
aacaggctat ttcttcatct acag 24

<210> 31

<211> 25

<212> DNA

<213> Mus musculus

<400> 31
ctcatcaatg tatcttatca tgtct 25

<210> 32

<211> 25

<212> DNA

<213> Mus musculus

<400> 32
ctcatcaatg tatcttatca tgtct 25

<210> 33

<211> 20

<212> DNA

<213> Mus musculus

<400> 33
agccgcggcc acaggaacag 20

<210> 34

<211> 19

<212> DNA

<213> Mus musculus

<400> 34
tggatgacat gacccatag 19

<210> 35

<211> 7

<212> PRT

<213> Homo sapiens

<400> 35

Met Asn Ser Arg Asn Lys Arg
1 5

<210> 36

<211> 60

<212> DNA

<213> Homo sapiens

<400> 36
atttgattct agaaggagga ataacatatg aacagccgta ataagcgtgc cgttcagggt 60

<210> 37

<211> 45

<212> DNA

<213> Homo sapiens

<400> 37
ccgcggatcc tcgagttaca gcagtttcaa tgcaccaaaa aatgt 45

<210> 38

<211> 17

<212> PRT

<213> Homo sapiens

<400> 38

Met Asp Tyr Lys Asp Asp Asp Asp Lys Lys Leu Asn Ser Arg Asn Lys
1 5 10 15

Arg

<210> 39

<211> 48

<212> DNA

<213> Homo sapiens

<400> 39
gacgatgaca agaagcttaa cagccgtaat aagcgtgccg ttcagggt 48

<210> 40

<211> 151

<212> PRT

<213> Mus musculus

<400> 40

Gln Asn Ser Ser Asp Lys Pro Val Ala His Val Val Ala Asn His Gln
1 5 10 15

Val Glu Glu Gln Leu Glu Trp Leu Ser Gln Arg Ala Asn Ala Leu Leu
20 25 30

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Ala Asn Gly Met Asp Leu Lys Asp Asn Gln Leu Val Val Pro Ala Asp
        35                  40                  45

Gly Leu Tyr Leu Val Tyr Ser Gln Val Leu Phe Lys Gly Gln Gly Cys
    50                  55                  60

Pro Asp Tyr Val Leu Leu Thr His Thr Val Ser Arg Phe Ala Ile Ser
65                  70                  75                  80

Tyr Gln Glu Lys Val Asn Leu Leu Ser Ala Val Lys Ser Pro Cys Pro
                85                  90                  95

Lys Asp Thr Pro Glu Gly Ala Glu Leu Lys Pro Trp Tyr Glu Pro Ile
            100                 105                 110

Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly Asp Gln Leu Ser Ala
        115                 120                 125

Glu Val Asn Leu Pro Lys Tyr Leu Asp Phe Ala Glu Ser Gly Gln Val
    130                 135                 140

Tyr Phe Gly Val Ile Ala Leu
145                 150
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<210> 41

<211> 1340

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (28)..(906)

<400> 41

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gtcgacccac gcgtccgatc ctgagta atg agt ggc ctg ggc cgg agc agg cga      54
                              Met Ser Gly Leu Gly Arg Ser Arg Arg
                              1               5

ggt ggc cgg agc cgt gtg gac cag gag gag cgc ttt cca cag ggc ctg      102
Gly Gly Arg Ser Arg Val Asp Gln Glu Glu Arg Phe Pro Gln Gly Leu
10                  15                  20                  25

tgg aca ggg gtg gct atg aga tcc tgc ccc gaa gag cag tac tgg gat      150
Trp Thr Gly Val Ala Met Arg Ser Cys Pro Glu Glu Gln Tyr Trp Asp
                30                  35                  40

cct ctg ctg ggt acc tgc atg tcc tgc aaa acc att tgc aac cat cag      198
Pro Leu Leu Gly Thr Cys Met Ser Cys Lys Thr Ile Cys Asn His Gln
            45                  50                  55

agc cag cgc acc tgt gca gcc ttc tgc agg tca ctc agc tgc cgc aag      246
Ser Gln Arg Thr Cys Ala Ala Phe Cys Arg Ser Leu Ser Cys Arg Lys
        60                  65                  70

gag caa ggc aag ttc tat gac cat ctc ctg agg gac tgc atc agc tgt      294
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Glu Gln Gly Lys Phe Tyr Asp His Leu Leu Arg Asp Cys Ile Ser Cys
    75                  80                  85

gcc tcc atc tgt gga cag cac cct aag caa tgt gca tac ttc tgt gag      342
Ala Ser Ile Cys Gly Gln His Pro Lys Gln Cys Ala Tyr Phe Cys Glu
90                  95                  100                 105

aac aag ctc agg agc cca gtg aac ctt cca cca gag ctc agg aga cag      390
Asn Lys Leu Arg Ser Pro Val Asn Leu Pro Pro Glu Leu Arg Arg Gln
                110                 115                 120

cgg agt gga gaa gtt gaa aac aat tca gac aac tcg gga agg tac caa      438
Arg Ser Gly Glu Val Glu Asn Asn Ser Asp Asn Ser Gly Arg Tyr Gln
            125                 130                 135

gga ctg gag cac aga ggc tca gaa gca agt cca gct ctc ccg ggg ctg      486
Gly Leu Glu His Arg Gly Ser Glu Ala Ser Pro Ala Leu Pro Gly Leu
        140                 145                 150

aag ctg agt gca gat cag gtg gcc ctg gtc tac agc acg ctg ggg ctc      534
Lys Leu Ser Ala Asp Gln Val Ala Leu Val Tyr Ser Thr Leu Gly Leu
    155                 160                 165

tgc ctg tgt gcc gtc ctc tgc tgc ttc ctg gtg gcg gtg gcc tgc ttc      582
Cys Leu Cys Ala Val Leu Cys Cys Phe Leu Val Ala Val Ala Cys Phe
170                 175                 180                 185

ctc aag atg agg ggg gat ccc tgc tcc tgc cag ccc cgc tca agg ccc      630
Leu Lys Met Arg Gly Asp Pro Cys Ser Cys Gln Pro Arg Ser Arg Pro
                190                 195                 200

cgt caa agt ccg gcc aag tct tcc cag gat cac gcg atg gaa gcc ggc      678
Arg Gln Ser Pro Ala Lys Ser Ser Gln Asp His Ala Met Glu Ala Gly
            205                 210                 215

agc cct gtg agc aca tcc ccc gag cca gtg gag acc tgc agc ttc tgc      726
Ser Pro Val Ser Thr Ser Pro Glu Pro Val Glu Thr Cys Ser Phe Cys
        220                 225                 230

ttc cct gag tgc agg gcg ccc acg cag gag agc gca gtc acg cct ggg      774
Phe Pro Glu Cys Arg Ala Pro Thr Gln Glu Ser Ala Val Thr Pro Gly
    235                 240                 245

acc ccc gac ccc act tgt gct gga agg tgg ggg tgc cac acc agg acc      822
Thr Pro Asp Pro Thr Cys Ala Gly Arg Trp Gly Cys His Thr Arg Thr
250                 255                 260                 265

aca gtc ctg cag cct tgc cca cac atc cca gac agc ggc ctt ggc att      870
Thr Val Leu Gln Pro Cys Pro His Ile Pro Asp Ser Gly Leu Gly Ile
                270                 275                 280

gtg tgt gtg cct gcc cag gag ggg ggc cca ggt gca taaatggggg           916
Val Cys Val Pro Ala Gln Glu Gly Gly Pro Gly Ala
            285                 290

tcagggaggg aaaggaggag ggagagagat ggagaggagg ggagagagaa agagaggtgg    976

ggagagggga gagagatatg aggagagaga gacagaggag gcagagaggg agagaaacag   1036

aggagacaga gagggagaga gagacagagg gagagagaga cagagaggaa gagaggcaga   1096

gagggaaaga ggcagagaag gaaagagaca ggcagagaag gagagaggca gagagggaga   1156

gaggcagaga gggagagagg cagagagaca gagagggaga gagggacaga gagagataga   1216
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gcaggaggtc ggggcactct gagtcccagt tcccagtgca gctgtaggtc gtcatcacct 1276

aaccacacgt gcaataaagt cctcgtgcct gctgctcaca gcccccgaga gcccctcctc 1336

ctgg 1340

<210> 42

<211> 293

<212> PRT

<213> Homo sapiens

<400> 42

Met Ser Gly Leu Gly Arg Ser Arg Arg Gly Gly Arg Ser Arg Val Asp
1 5 10 15

Gln Glu Glu Arg Phe Pro Gln Gly Leu Trp Thr Gly Val Ala Met Arg
20 25 30

Ser Cys Pro Glu Glu Gln Tyr Trp Asp Pro Leu Leu Gly Thr Cys Met
35 40 45

Ser Cys Lys Thr Ile Cys Asn His Gln Ser Gln Arg Thr Cys Ala Ala
50 55 60

Phe Cys Arg Ser Leu Ser Cys Arg Lys Glu Gln Gly Lys Phe Tyr Asp
65 70 75 80

His Leu Leu Arg Asp Cys Ile Ser Cys Ala Ser Ile Cys Gly Gln His
85 90 95

Pro Lys Gln Cys Ala Tyr Phe Cys Glu Asn Lys Leu Arg Ser Pro Val
100 105 110

Asn Leu Pro Pro Glu Leu Arg Arg Gln Arg Ser Gly Glu Val Glu Asn
115 120 125

Asn Ser Asp Asn Ser Gly Arg Tyr Gln Gly Leu Glu His Arg Gly Ser
130 135 140

Glu Ala Ser Pro Ala Leu Pro Gly Leu Lys Leu Ser Ala Asp Gln Val
145 150 155 160

Ala Leu Val Tyr Ser Thr Leu Gly Leu Cys Leu Cys Ala Val Leu Cys
165 170 175

Cys Phe Leu Val Ala Val Ala Cys Phe Leu Lys Met Arg Gly Asp Pro
180 185 190

Cys Ser Cys Gln Pro Arg Ser Arg Pro Arg Gln Ser Pro Ala Lys Ser
195 200 205

Ser Gln Asp His Ala Met Glu Ala Gly Ser Pro Val Ser Thr Ser Pro
210 215 220

Glu Pro Val Glu Thr Cys Ser Phe Cys Phe Pro Glu Cys Arg Ala Pro
225 230 235 240

Thr Gln Glu Ser Ala Val Thr Pro Gly Thr Pro Asp Pro Thr Cys Ala
245 250 255

Gly Arg Trp Gly Cys His Thr Arg Thr Thr Val Leu Gln Pro Cys Pro
260 265 270

His Ile Pro Asp Ser Gly Leu Gly Ile Val Cys Val Pro Ala Gln Glu
275 280 285

Gly Gly Pro Gly Ala
290

<210> 43

<211> 291

<212> PRT

<213> Homo sapiens

<400> 43

Met Ser Gly Leu Gly Arg Ser Arg Arg Gly Gly Arg Ser Arg Val Asp
1 5 10 15

Gln Glu Glu Arg Phe Pro Gln Gly Leu Trp Thr Gly Val Ala Met Arg
20 25 30

Ser Cys Pro Glu Glu Gln Tyr Trp Asp Pro Leu Leu Gly Thr Cys Met
35 40 45

Ser Cys Lys Thr Ile Cys Asn His Gln Ser Gln Arg Thr Cys Ala Ala
50 55 60

Phe Cys Arg Ser Leu Ser Cys Arg Lys Glu Gln Gly Lys Phe Tyr Asp
65 70 75 80

His Leu Leu Arg Asp Cys Ile Ser Cys Ala Ser Ile Cys Gly Gln His
85 90 95

Pro Lys Gln Cys Ala Tyr Phe Cys Glu Asn Lys Leu Arg Ser Pro Val
100 105 110

Asn Leu Pro Pro Glu Leu Arg Arg Gln Arg Ser Gly Glu Val Glu Asn
115 120 125

Asn Ser Asp Asn Ser Gly Arg Tyr Gln Gly Leu Glu His Arg Gly Ser
130 135 140

Glu Ala Ser Pro Ala Leu Pro Gly Leu Lys Leu Ser Ala Asp Gln Val
145 150 155 160

Ala Val Tyr Ser Thr Leu Gly Leu Cys Leu Cys Ala Val Leu Cys Cys
165 170 175

Phe Leu Val Ala Val Ala Cys Phe Leu Lys Met Arg Gly Asp Pro Cys
180 185 190

Ser Cys Gln Pro Arg Ser Arg Pro Arg Gln Ser Pro Ala Lys Ser Ser
195 200 205

Gln Asp His Ala Met Glu Ala Gly Ser Pro Val Ser Thr Ser Pro Glu
210 215 220

Pro Val Glu Thr Cys Ser Phe Cys Phe Pro Glu Cys Arg Ala Pro Thr
225 230 235 240

Gln Glu Ser Ala Val Thr Pro Gly Thr Pro Asp Thr Cys Ala Gly Arg
245 250 255

Trp Gly Cys His Thr Arg Thr Thr Val Leu Gln Pro Cys Pro His Ile
260 265 270

Pro Asp Ser Gly Leu Gly Ile Val Cys Gly Pro Ala Gln Glu Gly Gly
275 280 285

Pro Gly Ala
290

<210> 44

<211> 32

<212> PRT

<213> Homo sapiens

<400> 44

Met Ser Gly Leu Gly Arg Ser Arg Arg Gly Gly Arg Ser Arg Val Asp
1 5 10 15

Gln Glu Glu Arg Phe Pro Gln Gly Leu Trp Thr Gly Val Ala Met Arg
20 25 30

<210> 45

<211> 37

<212> PRT

<213> Homo sapiens

<400> 45

Ser Cys Pro Glu Glu Gln Tyr Trp Asp Pro Leu Leu Gly Thr Cys Met
1 5 10 15

Ser Cys Lys Thr Ile Cys Asn His Gln Ser Gln Arg Thr Cys Ala Ala
20 25 30

Phe Cys Arg Ser Leu
35

<210> 46

<211> 38

<212> PRT

<213> Homo sapiens

<400> 46

Ser Cys Arg Lys Glu Gln Gly Lys Phe Tyr Asp His Leu Leu Arg Asp
1 5 10 15

Cys Ile Ser Cys Ala Ser Ile Cys Gly Gln His Pro Lys Gln Cys Ala
20 25 30

Tyr Phe Cys Glu Asn Lys
35

<210> 47

<211> 57

<212> PRT

<213> Homo sapiens

<400> 47

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Gly Glu Val Glu Asn Asn Ser Asp Asn Ser Gly Arg Tyr Gln Gly Leu
20 25 30

Glu His Arg Gly Ser Glu Ala Ser Pro Ala Leu Pro Gly Leu Lys Leu
35 40 45

Ser Ala Asp Gln Val Ala Val Tyr Ser
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<210> 48

<211> 21

<212> PRT

<213> Homo sapiens

<400> 48

Thr Leu Gly Leu Cys Leu Cys Ala Val Leu Cys Cys Phe Leu Val Ala
1 5 10 15

Val Ala Cys Phe Leu
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<210> 49

<211> 106

<212> PRT

<213> Homo sapiens

<400> 49

Lys Met Arg Gly Asp Pro Cys Ser Cys Gln Pro Arg Ser Arg Pro Arg
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Gln Ser Pro Ala Lys Ser Ser Gln Asp His Ala Met Glu Ala Gly Ser
20 25 30

Pro Val Ser Thr Ser Pro Glu Pro Val Glu Thr Cys Ser Phe Cys Phe
35 40 45

Pro Glu Cys Arg Ala Pro Thr Gln Glu Ser Ala Val Thr Pro Gly Thr
50 55 60

Pro Asp Thr Cys Ala Gly Arg Trp Gly Cys His Thr Arg Thr Thr Val
65 70 75 80

Leu Gln Pro Cys Pro His Ile Pro Asp Ser Gly Leu Gly Ile Val Cys
85 90 95

Gly Pro Ala Gln Glu Gly Gly Pro Gly Ala
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<210> 50

<211> 32

<212> DNA

<213> Homo sapiens

<400> 50
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<212> DNA

<213> Homo sapiens

<400> 51
tctccgcggc cgcgctgtag accagggcca cctg 34

<210> 52

<211> 6

<212> PRT

<213> Homo sapiens

<400> 52

Gly Ala Leu Lys Leu Leu
1 5

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(74) Agents: ODRE, Steven, M. et al.; Amgen Inc., One Amgen Center Drive, Mail Stop 27-4-A, Thousand Oaks, CA 91320-1799 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

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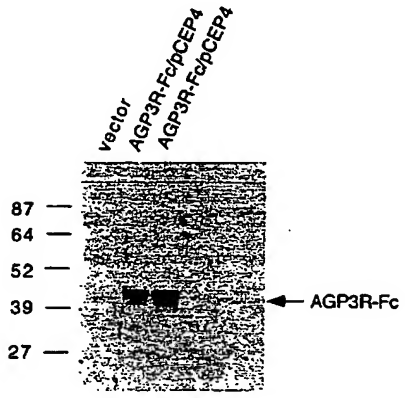
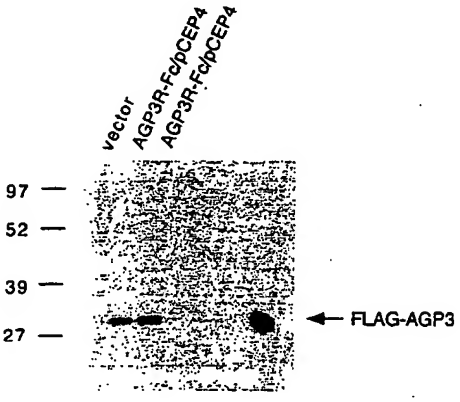
[Continued on next page]

(54) Title: FUSION RECEPTOR FROM TNF FAMILY





(57) Abstract: A member of the tumor necrosis factor family and its receptor are described. This member is primarily expressed in B cells and its expression correlates to increases in the number of B cells and immunoglobulins produced. The natural, preferred human ortholog is here called AGP-3R. The protein is a type III transmembrane protein and has an amino terminal extracellular domain, a transmembrane domain, and a carboxy terminal intracellular domain. AGP-3R-related proteins of the invention may be membrane-associated or in soluble form, recombinantly produced or isolated after natural production. The invention provides for nucleic acids encoding such AGP-3R-related proteins, vectors and host cells expressing the polypeptides, and methods for producing recombinant proteins. Antibodies or fragments thereof that specifically bind the proteins are also provided.

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Published:
— *with international search report*

(88) Date of publication of the international search report:
25 April 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 01/04568

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C12N15/62 C07K14/715 C07K19/00 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, SEQUENCE SEARCH, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 39361 A (ST JUDE CHILDRENS RES HOSPITAL) 11 September 1998 (1998-09-11) page 73; claims 7,8,19; figures SEQ.ID.6, page 4, line 32 - line 33 page 24, line 20 -page 25, line 29 page 6, line 21 -page 6, line 25 page 58, paragraph 1 page 19, paragraph 4 page 8, paragraph 2; claims 14,15 --- -/--	1-4, 12-18

[X] Further documents are listed in the continuation of box C

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 August 2001	27 November 2001 (27.11.01)

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gurdjian, D

3

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BUELOW VON G-U ET AL: "NF-AT activation induced by a CAML-interacting member of the tumor necrosis factor receptor superfamily" SCIENCE, AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, US, vol. 278, no. 5335, 3 October 1997 (1997-10-03), pages 138-141, XP002165404 ISSN: 0036-8075 abstract	1-4, 12-18
P,X	WO 00 40716 A (ZYMOGENETICS INC) 13 July 2000 (2000-07-13) page 149 -page 150; example 6	1-4, 12-18
P,X	WO 00 58362 A (HUMAN GENOME SCIENCES INC ;BAKER KEVIN (US); RUBEN STEVEN M (US);) 5 October 2000 (2000-10-05) claims 1-20; example 1	1-6, 12-18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 01/04568

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 19,20,29,30 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-3, 10-20 partly and 4-6

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-3 ,10-20 partly and 4-6

   Chimeric constructs comprising amino acid sequence seq.id.45 and/or 46 and a Ig Fc region , corresponding nucleic acid , expression vector , host cell , pharmaceutical composition and method of modulating AGP-3 .

2. Claims: 1-3 ,7,10-20 partly and 8

   Chimeric constructs comprising amino acid sequence seq.id.45 and/or 46 and a water soluble polymer , like poylethylene glycol , corresponding nucleic acid , expression vector , host cell , pharmaceutical composition and method of modulating AGP-3 .

3. Claims: 1-3 ,7,10-20 partly and 9

   Chimeric constructs comprising amino acid sequence seq.id.45 and/or 46 and a carbohydrate , like dextran , corresponding nucleic acid , expression vector , host cell , pharmaceutical composition and method of modulating AGP-3 .

4. Claims: 21-30

   Chimeric construct comprising an antibody sequence in which one or more amino acids from antibody variable domains or CDR regions have been replaced with amino acid sequence selected from seq.id.45 and 46 , corresponding nucleic acid , expression vector , host cell , pharmaceutical composition and method of modulating AGP-3 .

INTERNATIONAL SEARCH REPORT

.ormation on patent family members

Interr nal Application No
PCT/US 01/04568

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9839361 A	11-09-1998	US 5969102 A	19-10-1999
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		EP 0964874 A1	22-12-1999
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		EP 1141274 A2	10-10-2001
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		WO 0040716 A2	13-07-2000
WO 0058362 A	05-10-2000	AU 3002800 A	14-09-2000
		AU 3633000 A	16-10-2000
		WO 0050597 A2	31-08-2000
		WO 0058362 A1	05-10-2000